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Bul. M. Lalića 1, 81000 Podgorica, Crna Gora (Montenegro), P.Box 97,
Tel.: +382 20 268434; +382 20 268437; Fax: +382 20 268432
Web: www.agricultforest.ac.me; E-mail: agricultforest@ac.me

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CONTENT

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Radovan KASARDA,
Nina MORAVČÍKOVÁ, Ondrej KADLEČÍK [1]

GENETIC STRUCTURE OF WARMBLOOD HORSES ON MOLECULAR-GENETIC LEVEL

SUMMARY

The aim of this study was to evaluate the genetic structure and potential loss of diversity within three horse breeds: Lipizzan (42), Furioso (38), and Nonius (18) on molecular-genetic level. The genotyping data were obtained from in total 98 individuals presenting the genepool of analysed breeds in Slovakia. The values of gene diversity (0.72) as well as heterozygosity (0.89) showed the prevalence of heterozygotes within each population. The negative value of F_{IS} index (-0.25) pointed, in particular, to good level of diversity and balanced proportion of homozygous and heterozygous animals in populations, i.e. HWE equilibrium. The Shannon's information index (I=1.41) confirmed sufficient level of variability mainly in order to maintain the current state of biodiversity for future generation. The genetic distances on intra- and inter-population level revealed clearly distribution of individuals into the three separate genetic clusters. The highest genetic similarity was found between Nonius and Furioso breeds (DA=0.16). However, the pairwise F_{ST} values and PCA analysis were not fully sensitive to detect the genetic differences among those breeds. We were able to detect only two genetic clusters, when Furioso and Nonius were linked together. This can be explained mainly by the use of English Thoroughbreds stallions and mares in building up process of both breeds. Considering that the results related to the state of diversity in presented breeds are correct, they can be used as basis for the further progress in breeding programs which is necessary to prevent the loss of heterozygosity and increase of inbreeding.

Keywords: AnGR, diversity, genetic differentiation, horse breeding, microsatellites variability.

INTRODUCTION

Changes of conditions in production systems as well as significant competition among breeds create expectation of risk, which will negatively influence surviving of livestock species including horses. Within this context some breeds strengthen their position and expanded to different countries, while others, not based on commercialization, started to decrease their number and become endangered (Kadlečík and Kasarda, 2014).

[1]Radovan Kasarda, (corresponding author: radovan.kasarda@uniag.sk), Nina Moravčíková, Ondrej Kadlečík, Department of Animal Genetics and Breeding Biology, Slovak University of Agriculture in Nitra, SLOVAKIA

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In recent years, a slight increase of the global equine population size has been recorded. The world's equine population is estimated at 65 million according to the Food and Agriculture Organization of the United Nations (FAOSTAT). Unfortunately, this positive trend of population size so completely does not apply in Slovakia. The horse breeding and its improvement in our region was negatively influenced mainly by privatization mostly followed by bankruptcy of purebred animals breeding in stud farms in the nineties. Currently, the horse population reach total number around 16,600 only, placing the Slovak Republic to the last position in the horse population size in the middle European region. The development of particular populations in Slovakia is affected mainly by the decline in mares' proportion and also due to negative changes in population age structure (Halo et al., 2008; Zöldág, 2008; Buleca et al., 2010).

Despite the low effective population size the horse breed variability is colourful. Lisáková (2009) indicates that in the 2008 11,068 horses of 27 different breeds were bred in Slovakia. But given the current state across warmblood horses in Slovakia, populations of Furioso (number of active breeding horses in Stud book on the level 30 stallions and 180 mares, while in reproduction is involved only 60 mares) and Lipizzan (with total number of mares 80 and number of stallions 13) breeds are considered as endangered by the loss of genetic diversity. In addition, population of Nonius horses with total number of active stallions on level 8 and 110 mares (but only 30 is reproductive active) is regarded as critically endangered (ZCHKS, 2017). Mainly for this reason, the Ministry of Agriculture of the Slovak Republic is planning to increase support of the horse breeding namely in the branches of draught work in agriculture and forestry including agrotourism and also in sport utilization in horse riding and hipotherapy. In the reaction to the changes in social and political systems the state of "certified breeding organization" in the area of horse breeding only 3 organizations were assigned by Ministry of Agriculture: Slovak Horse Breeding Association, National Stud in Topoľčianky and Turf Directory in Bratislava (Buleca et al., 2010).

The analysis of genetic diversity as well as the loss of heterozygosity of horse populations in Slovakia have been evaluated mainly based on the pedigree data (Pjontek et al., 2010; Hazuchová et al., 2013). However, the current availability of different type of genetic markers allows more precise examination compared to pedigree analysis and offer advantages that are particularly appropriate for future conservation of AnGR (Petersen et al. 2013). One of the most commonly used markers are microsatellite loci. Genetic characterization based on microsatellites analysis proved to be a reliable tool to quantify genetic variation within and among breeds thereby contributing to the orientation of conservation strategies (Rukavina et al., 2015).

The aim of this study was to analyse genetic structure of populations and degree of diversity loss within three horse breeds (Lipizzan, Furioso, and Nonius) that belong currently to most endangered warmblood horse populations in Slovakia.

MATERIAL AND METHODS

The genetic variability was evaluated on both intra- and inter-population levels based on the genotyping data that was collected from a total of 98 individuals presenting the genepool of three breeds: Lipizzan (N=42), Furioso (N=38), and Nonius (N=18) in Slovakia. The genotype database of individuals was prepared by using overall 13 microsatellite systems (*AHT4*, *AHT5*, *ASB2*, *HMS1*, *HMS2*, *HMS3*, *HMS6*, *HMS7*, *HTG10*, *HTG4*, *HTG6*, *HTG7*, and *VHL20*), routinely used for paternity testing.

The potential loss of genetic diversity within breeds was measured based on following parameters: mean number of alleles (*MNA*), effective allele number (N_e), observed heterozygosity (H_o), expected heterozygosity mostly referred to as gene diversity (H_e), and Shannon's index (*I*) using the Genalex version 6.1 (Peakall and Smouse, 2012). The significance of HWE departure (Hardy-Weinberg equilibrium) reflecting the differences between observed and expected genotype frequencies were tested by Chi-square test. The amount of inbreeding-like effect within population (F_{IT} or *F*), among individuals in population (F_{IS} or *f*), and among individuals within subpopulations (F_{ST} or Θ) was measured according to the method described by Weir and Cockerham (1984) using FSTAT software (Goudet, 2002).

The hierarchical distribution of molecular variance was tested by ANOVA statistical model implemented in the Arlequin v3.5 (Excoffier et al., 2005). An analysis of molecular variance was performed using 10,000 permutations. The genetic differentiation were calculated on inter-population level based on Wright's F_{ST} index and on intra-population level by using Nei's standard distance noted as D_A (Nei et al., 1983). Nei's D_A distance assumes that the differentiation arises due to mutations and genetic drift. It is known that this indices give more reliable results particularly for microsatellite data. The population genetic structure was tested based on the principal component analysis (PCA) using implemented in R package *adegenet* 1.3-0 (Jombart and Ahmed, 2011).

RESULTS AND DISCUSSION

Generally, in view of AnGR conservation proportion of genetic diversity within each of analysed populations can be regarded as sustainable and reflecting only slight loss of total genetic variability. Significant differences indicated potential loss of variability within populations was found only between mean number of allele (5.231±0.426) and effective allele number (4.007±0.299). But the proportion of alleles with frequency higher than 0.05 was comparable with observed effective allele numbers (Fig. 1). The number of private alleles characterizing genetic uniqueness of analysed breeds ranged from 0.385 (Nonius) to 0.923 (Furioso) that signalized only relative low degree of genetic differentiation across analysed populations. Expected heterozygosity in range from 0.650 (Lipizzan) to 0.755 (Furioso) indicated sufficient proportion of heterozygote individuals. Similarly Wright's F_{IS} index confirmed higher proportion of heterozygotes in each population (-0.246±0.032). The Shannon's

information index (1.41±0.049) confirmed sufficient level of variability mainly in order to maintain the current state of biodiversity for future generation (Fig. 1). The obtained level of genetic diversity within breeds is comparable to the results found in other warmblood horses, including for example Haflinger (Vostrý et al., 2015) or Old Kladruber horses (Kasarda et al., 2016).

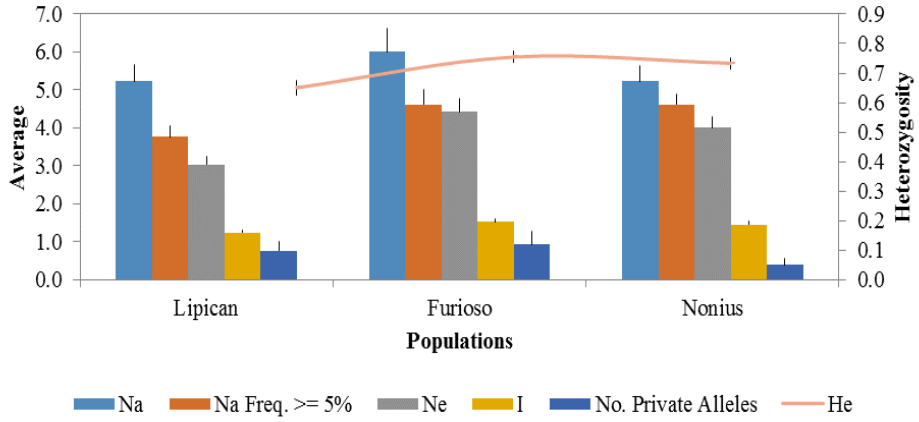

Figure 1. Allelic pattern across populations.

The principal component analysis was performed to describe the highest proportion of variance in dataset. The retained 96 PCA axes in analysis corresponded to more than 90% of conserved variance within metapopulation. The highest proportion of variance was explained by first two principal components that were subsequently used to visualization of genetic relationship among individuals (Fig. 2).

Based on first principal component we were able to describe 2.33% of variance and second component represented overall 1.13% of variance. Figure 2 clearly shows the division of individuals into the clusters according their origin mainly with respect to historical development of each breed. As we can see on the Figure 2 Nonius and Furioso populations were linked together, whereas Lipizzan horses formed separate cluster. In case of Nonius and Furioso populations this indicated higher degree of genetic similarity between them that was also proved by Nei's genetic distances and F_{ST} index.

The matrix constructed based on Nei's genetic distance showed that the populations of Nonius and Furioso can be regarded as genetically more similar than any other pair-wise comparison with Lipizzan (D_a=0,164). The highest genetic distance was observed between Lipizzan and Nonius populations (D_a=0,336). On the individual level the genetic distance matrix revealed three separate clusters. Figure 3 shows distribution of individuals into clusters reflecting genetic background of each breed. Based on this we can conclude that the Nei's approach was more sensitive to analysis of genetic relationship within and between horse populations than PCA analysis.

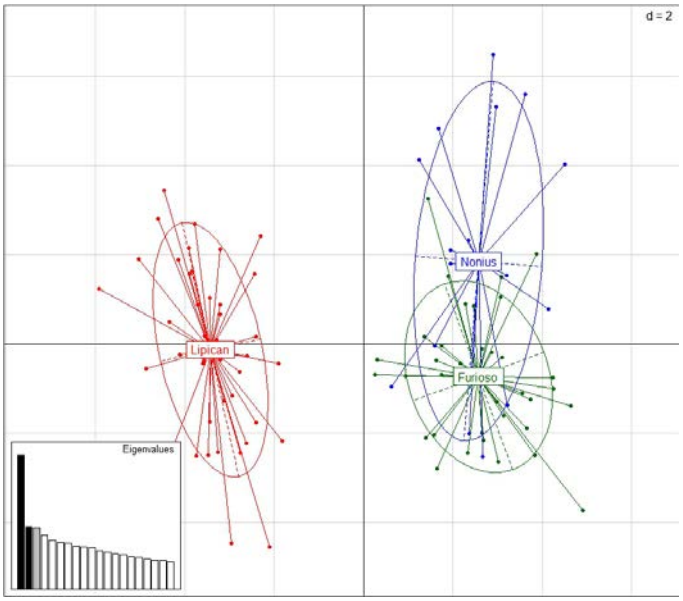

Figure 2. Genetic structure of population based on PCA analysis.

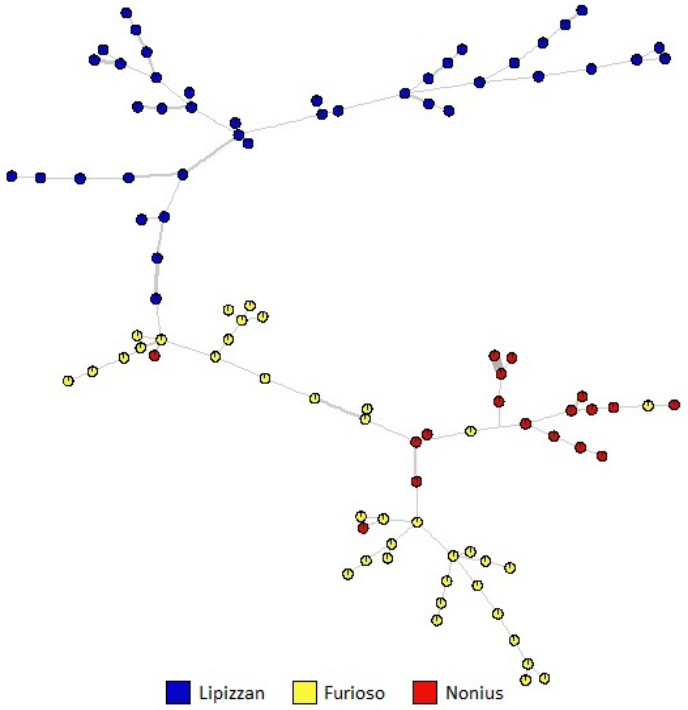

Figure 3. Population structure presenting relationship among individuals based on Nei's Da genetic distances.

One of the reasons can be relatively small sample size of Nonius population as well as the proportion of missing data. This bias can be reduces by applying approaches that utilize also correction for sample size. On the other hand more logically explanation of our results is fact that the Nonius and Furioso breeds have common historical origin. Both of breeds have been bred in Mezöhegyes Stud Farm. In the founder population of Nonius breed the maternal lines was created by using mares of Spanish-Neapolitan origin and characteristic of these mares are still evident in the breed today. The progenitor of the Nonius breed was an Anglo-Norman stallion Nonius senior (Lukáč, 2010). Later, to correct faults that had manifested in the Nonius breed, blood of English Thoroughbreds was used. The Furioso horses were created by using foundations sires Furioso (English Thoroughbred stallion) and North Star. The mares were mostly Nonius. Furioso have been also crossed by other English Thoroughbreds stallions to produce animals highly qualified for sporting purposes (ZCHKS, 2017).

CONCLUSIONS

Development of molecular genetic techniques provides now opportunity for increase of selection strategies and livestock breeding. The spectrum of genetic markers utilization including microsatellites is huge and is still expanding. The microsatellites are widely used particularly in genome mapping, phylogenetic studies, forensic analyses, genotyping of individuals in population, determination of paternity, estimation of effective population size a population structure, and analysis of genetic diversity.

Considering that the results related to the state of diversity in presented breeds are correct, they can be used as basis for the further progress in breeding programs which is necessary to prevent the loss of heterozygosity and increase of inbreeding. Conservation of AnGR diversity allows to protect its as cultural heritage for future generation.

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Bojan ĐURIN, Anita PTIČEK SIROČIĆ, Nikola SAKAČ[1]

MODELLING OF METEOROLOGICAL PARAMETERS FOR THE PURPOSE OF SIZING OF THE SOLAR PHOTOVOLTAIC IRRIGATION SYSTEMS

SUMMARY

Solar photovoltaic (PV) irrigation systems are usually sized by using measured or modelled meteorological data, such as solar radiation, air temperature and precipitation during a year or a certain period. Experiences during sizing, as well as in the use of such systems, have shown certain discrepancies during the year, despite the available data such as annual cloud coverage, number of sunny days, etc. In such cases, the usual statistical parameters as well as trend analysis are not reliable tools for predicting deviations of the PV irrigation system operation.

The justification of using the Rescaled Adjusted Partial Sums (RAPS) method has been researched in his paper, in order to determine the existence of such deviations in the given time series (i.e. their deviations or fluctuations) of the solar radiation intensity, air temperature and precipitation. In this way the existence of certain irregularities of the given time series, i.e. input quantities of solar radiation intensity, air temperature and precipitation amounts were determined. This defined the period of the year in which special attention had to be paid to the operational functioning of the PV irrigation systems. The presented methodology was shown on real examples on several locations in the Republic of Croatia where PV irrigation systems are planned to be built. This methodology will certainly contribute to the achieving of systematic sustainability of the PV irrigation systems.

Keywords: Meteorological parameters, RAPS method, Solar photovoltaic energy, Irrigation system, Time series

INTRODUCTION

Climate changes are highly related to all meteorological parameters. Air temperature and insolation intensity are main input parameters for the sizing of the solar photovoltaic (PV) irrigation system. Additionally, rainfall enriches the natural watercourses or aquifer, and has an impact on the PV irrigation system working parameters. These parameters, i.e. their input values play an important role in sizing that kind of systems. Attention should be focused on the possible seasonal or even weekly value deviations or fluctuations, as well as irregularities in time series of particular input data. The mentioned input parameters have a

[1]Bojan Đurin, (corresponding author: bojan.djurin@gfv.hr), Anita Ptiček Siročić, Nikola Sakač, Faculty of Geotechnical Engineering, University of Zagreb, CROATIA.
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stochastic nature; considering the climate variability, the PV irrigation system could be oversized or undersized.

Most common approaches in the analysis of changes in meteorological parameters are based on defining and analysis of their time series trends. Since meteorological parameters depend on climate changes, time series trends are not reliable and could lead to wrong conclusions about the analyzed characteristic of the parameters. Using the time series analysis, except determining the general time series trend, it is possible to determine more detailed fluctuations within the examined time series (Bonacci, 2010). For that purpose, a RAPS (Rescaled Adjusted Partial Sums) method has been used in this paper (Garbrecht and Fernandez, 1994; Bonacci et al, 2008). The RAPS method has been widely used in hydrological and meteorological parameters analysis. Bonacci, 2010, used the RAPS method for the time series analysis of the average annual air temperature measured at 26 meteorological stations in Croatia. Furthermore, Bonacci et al, (2008) and Bonacci et al (2009), used RAPS method for the analysis of the flow and temperature of the Danube River at Bratislava (Slovakia) and for the analysis of the temperature of the Danube River in Croatia. Lojen et al (2009), used the RAPS method to determine the climate changes impact on the limestone layers at the Krka River (Croatia) basin. The RAPS method is mostly used for the flow analysis of watercourses and analysis of their hydrological regimes. If within the time series of meteorological parameters the presence of subseries is determined, it is easier to focus on a certain part of the original (default) time series. In this way, it is possible to determine the reasons causing the appearance of subseries.

MATERIAL AND METHODS

Definition of the RAPS method

The RAPS method is based on the time series analysis by the usage of the deviation sum curve. Graphical presentation of the RAPS method is suitable since it provides overcoming of the small systematical and random changes, errors and variability in the analyzed time series. Graphical presentation of the RAPS method indicates the existence of subseries with similar characteristics, larger number of trends, sudden value changes, irregular fluctuations, existence of periodicity of the analyzed time series, etc. The RAPS method is defined through the following expression:

$$RAPS_k = \sum_{t=1}^{k} \frac{Y_t - \overline{Y}}{S_Y} \tag{1}$$

Where $\overline{Y}$ is average value of the considered time series, S_y is standard deviation of the same series, n is number of data in time series, k is summation counter (k = 1, 2, 3…, n), (Bonacci, 2010). Graphical presentation of the $RAPS_k$ values obviously and without any doubt points to existence regularities in the analyzed parameters (Y_t) fluctuations. When the existence of the subseries

within the main series has been determined, the next step is to determine (as a general rule) the linear trends of the subseries.

Case study

In this paper two potential locations for building of the PV irrigation system have been analyzed. Both locations are in the North West area of Croatia. The first location was located in Sveti Ilija, the city of Varaždin; the second was located in Domašinec, Međimurje County. The Sveti Ilija location was planned for building of the irrigation system at the football pitch. The Domašinec location was planned for building of the irrigation system for the family farm holding. RAPS method was applied on time series of the total average daily insolation E_S on the inclined surface of 15°, oriented to the South (SODA, 2017), average daily air temperature T_a (DHMZ, 2017a) and total daily precipitation P for the year 2015 (DHMZ, 2017b). The mentioned parameters with calculated trends are presented in Figure 1-3.

Negligible low values of the coefficient of determination (R^2) show that definition of a linear trend (as mostly used trend) was not acceptable (Figure 1-3). From these figures it could be concluded that definition of any trend was not acceptable, due to the stochastic nature of the input parameters. This is a motivation for the application of a new method which could determinate the irregularities i.e. functional dependencies in input time series (like RAPS method).

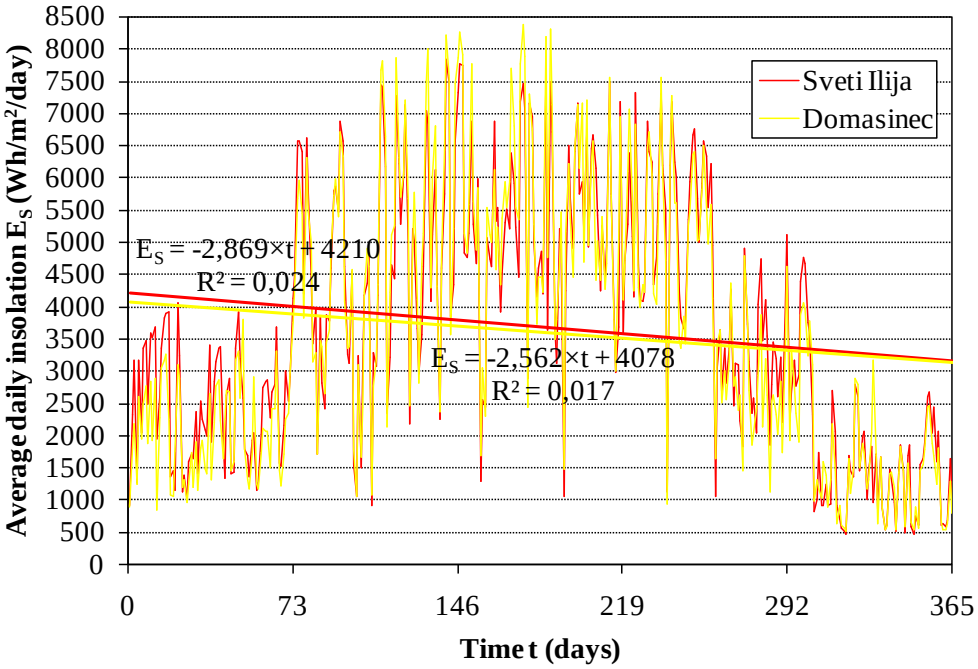

Figure 1. Average daily insolation for the Sveti Ilija and Domašinec.

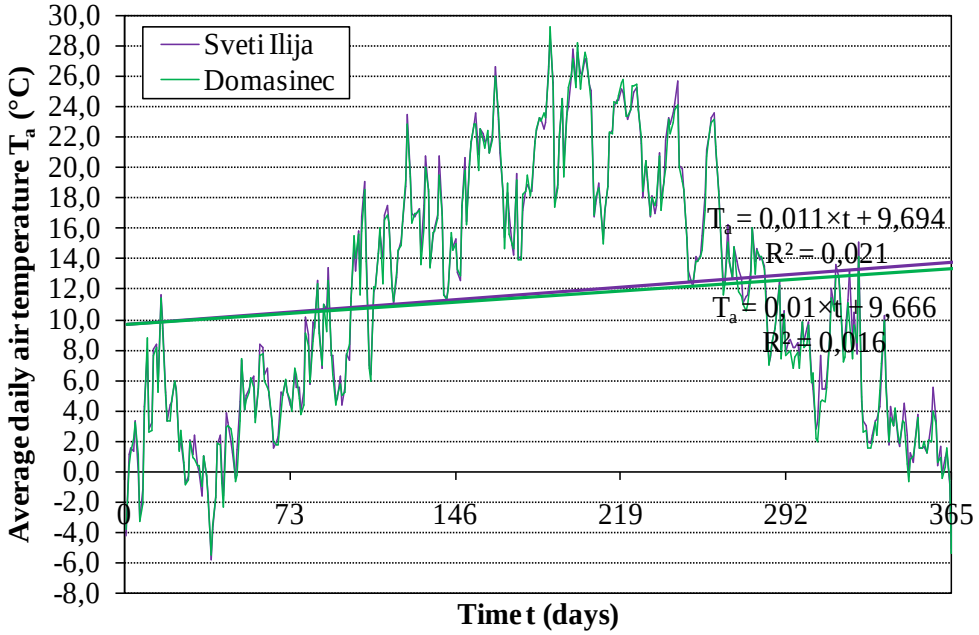

Figure 2. Average daily air temperature for the Sveti Ilija and Domašinec.

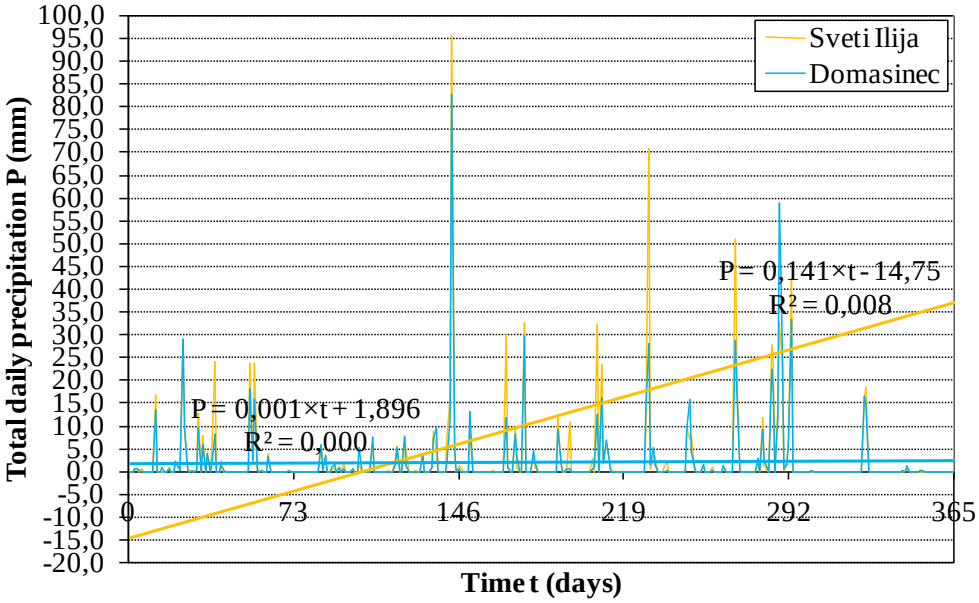

Figure 3. Total daily precipitation for the Sveti Ilija and Domašinec.

RESULTS AND DISCUSSION

Figure 4 presents RAPS values obtained by using Eq. 1 in the example of the average daily insolation E_S series.

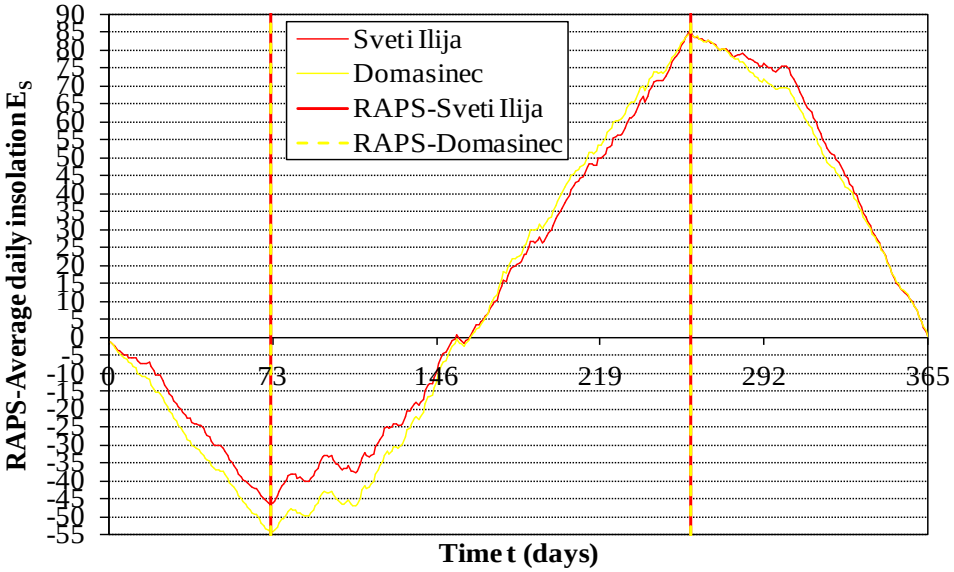

Figure 4. RAPS values for the average daily insolation.

Since Figure 3 shows the absence of continuity of the total daily precipitation for both sites (given the large number of days without any recorded precipitation), the application of the RAPS method is not acceptable due to discontinuity on both locations. Figures 5 and 6 present a calculated subseries for the average daily insolation E_S and average daily air temperature T_a.

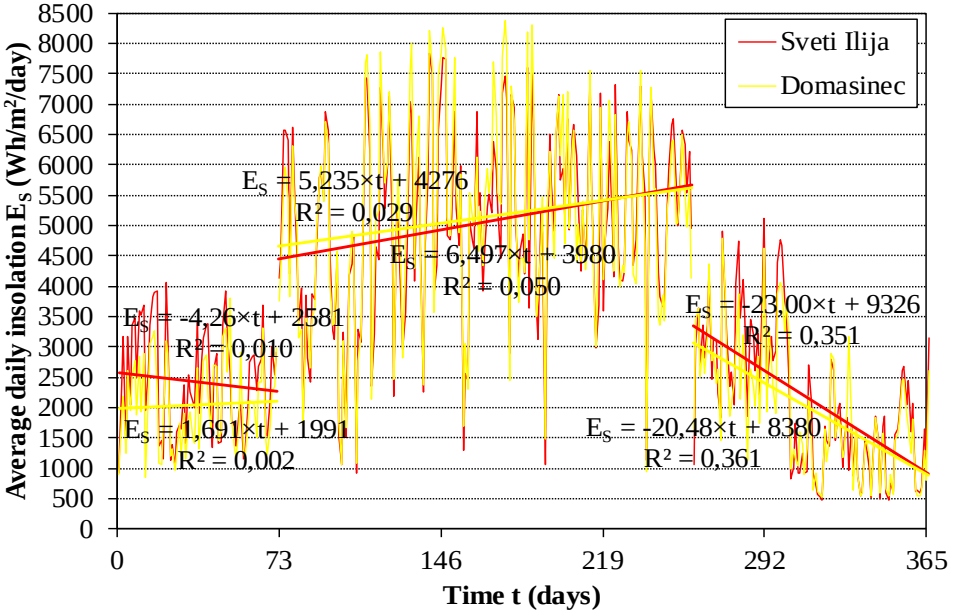

Figure 5. Subseries for the average daily insolation for the Sveti Ilija and Domašinec.

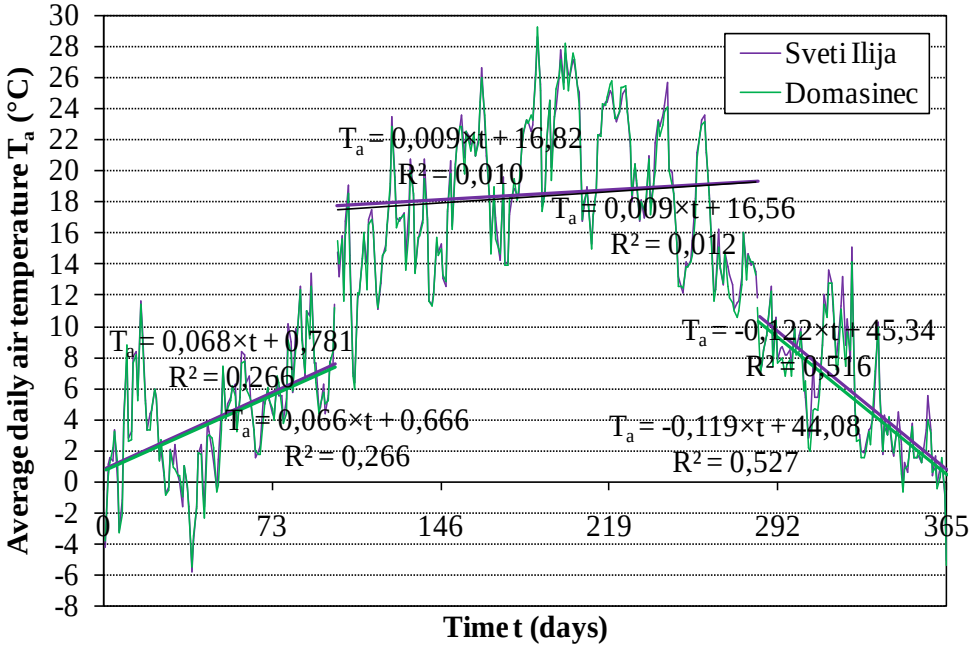

Figure 6. Subseries for the average daily air temperature for the Sveti Ilija and Domašinec.

In Figures 5 and 6, three characteristic periods (three new subseries) can be seen, bordered by the beginning and the ending of a summer season, for both locations. Due to the proximity of both sites, the subseries show strong overlapping. Even though the coefficients of the determination had low values with no significant functional dependence, there are regularities in series deviations.

This shows that in summer time (higher insolation, more water demand) a focus should be on the reliability of a PV irrigation system operational work. In this period of the year deviations in insolation and temperature are higher, compared to winter and spring time.

The PV irrigation system operational work directly depends on this. To increase the efficiency of the PV irrigation system, it is recommended to store water into the reservoirs or to use generators for producing electric energy, to use solar batteries, and to connect to classical electric energy grid.

The input parameters should be considered within the summer period, independently from the sizing method used. This is also supported by the unequal distribution of precipitation, shown in Figure 4, where there are visible extremes in the form of minimum but also maximum precipitation for both locations. Additionally, uneven precipitation distribution (Figure 4), i.e. maximums or minimums support the previous statement for both locations.

CONCLUSIONS

The RAPS method was employed to analyze the time series of the climate input parameters for sizing the PV irrigation systems. It was possible to determine the subseries where deviations were determined. During sizing of the PV irrigation systems it is necessary to focus on specific (or characteristic) time period according to possible deviations and fluctuations in system operational work. This is the result of stochastic and unpredictable nature of the input climate parameters.

RAPS method should be used in the long time period (ten years or more), as well for every particular year, due to a large number of usable data. This preliminary investigation presents an important base, but also a motivation for further investigation related to this interesting and up-to-date issue in irrigation.

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Fatma ACHEUK, Wassima LAKHDARI, Abderramene DAHLIZ, Khemais ABDELLAOUI, Manel MOUKADEM, Souhila ALLILI[1]

TOXICITY, ACETHYLCOLINESTERASE AND GLUTATHIONE S-TRANSFERASE EFFECTS OF HALOCNEMUM STROBILACEUM CRUDE EXTRACT AGAINST TRIBOLIUM CASTANEUM

SUMMARY

Halocnemum stobilaceum (Chenopodiaceae) is one of many halophyte plants from Algerian Sahara. In this study, we investigate the phytochemical composition and the insecticidal activity of the crude ethanolic extract of this plant against adults of the red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae). Te effect of this extract on the detoxification enzyme Glutathione S-transferase (GST) and the neuroenzyme Acethylcolinesterase (AChE) was also investigated. The repellent effect was evaluated, at the concentration of 1000 µg/ insect, using the preferential zone method on blotting paper. The insecticidal effect was investigated by testing 5 doses: 100, 200, 400, 800 and 1000 µg/insect.

The obtained results show that *H. stobilaceum* is very rich in saponins, gallic tannins, flavonoids, antocyans, coumarins and alkaloids. The plant is poor in irridoids. On insecticidal level, the extract tested at dose 1000 µg/insecte has a good repellent effect on adults of *T. castaneum*. The repulsively rate calculated after two hours of exposure was 60%. The extract was toxic too. Indeed, the five tested doses caused mortalities of 15, 33.3, 41.6, 48.3 and 70%, respectively, after 6 hours of exposure. The highest dose (1000 µg/insect) generated 100 deaths after 96 h of exposure. The LD_{50} calculated 24 h after treatment was 225.4 µg/insect. Furthermore, the extract of this plant, at the concentration of 1000 µg/insect, inhibited acetylcholinesterase (AChE) activity. The obtained results suggest that extracts of this plant can be used to protect the stored products against insect secondary pest.

Keywords: Crude ethanolic extract, Enzymatic assays, *Halocnemum stobilaceum*, Repellency, Toxicity, *Tribolium castaneum*

INTRODUCTION

Grains and milling products constitute a major part of the daily of human and animal populations among. The most important risk associated with flour and

[1]Fatma Acheuk (corresponding author: fatma.acheuk@yahoo.fr), Manel Moukadem, Souhila Allili, Laboratory of Valorization and Conservation of Biological Resources"Valcore", Department of Biology, Faculty of Sciences, University of Boumerdes, Boumerdes, 35000 ALGERIA; Wassima Lakhdari, Abderramene Dahliz, National Institute of Agronomic Research, Station of Sidi Mehdi, Touggourt, ALGERIA; Khemais Abdellaoui, Department of Biological Sciences and Plants Protection, High Institute of agriculture, Chott Mariem, Sousse University, TUNISIA

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cereal product consumption is insect contamination, which is an important quality control problem of concern milling industries (Taban et al., 2017). Currently, there are different kinds of preventive and curative control measures to get protection from this pest and chemical pesticides are one of them (Habib and Karim, 2015).These insecticides bring about such serious problems as contamination of the environment, lethal effects in non-targeted organisms which can lead to a failure of biological control programs and insect resistance. Toxic residues on stored grain for human consumption are other problems related to chemical pesticides (Rajendran and Sriranjini, 2008). However, with growing evidence that many conventional pesticides can adversely affect the environment and the human safety, requirement for safer means of pest management have become crucial (Rozman et al., 2007).One such alternative is the use of botanical insecticides (Baskar et al., 2009).

In the co-evolution of plant-insect interactions, plants have been able to synthesize a wide range of products to defend themselves against insect attacks, including primary metabolites (e.g. proteinase, amylase inhibitor and lectins) and secondary metabolites (e.g. alkaloids, tannins and rotenoids) (Zanini-Martins et al., 2012). Indeed, plants may provide potential option to currently used insect-control agents because they constitute a rich source of bioactive chemicals. Plant secondary compounds have been, than, the subject of investigation for the past 20 years in an effort to discover new sources of botanical insecticides, repellents and antifeedants (Akhtar and Isman, 2004).The main advantage of phytochemical insecticides is that they could be prepared easily by farmers, small-scal industries and are potentially less expensive (Nikkon et al., 2009).

In Algeria, research on the discovery and exploitation of natural resources for the development of botanical insecticides, for pest control, has been undertaken for several years (Acheuk et al., 2012 ; 2017 a & b; Acheuk and Doumandji-Mitiche, 2013 ; Dane, 2016).

Halophytes are an important group of plants and play key roles in the ecosystem. While they have been used by local communities for millennia, their full potential is still untapped. Some halophytes are now being harvested commercially to obtain gums, oils and resins for production of household goods, food processing, and heavy industrial applications. Other halophytes are well known for their bioactive derivatives and are essential ingredients for pharmaceuticals, agricultural pesticides, traditional medicines and natural cosmetics (Al-Oudat and Qadir, 2011).

Halocnemum strobilaceum is a halophilic *Chenopodiaceae* plant of saline and marshy areas, it's grows in damp salt soil (salinity > 90 dS/m). The plant was grazed by sheep and camels, but Bedouins believe that overfeeding on this plant causes lung disease in camels (Al-Oudat and Qadir, 2011).

Currently, no data concerning the biological or the insecticidal effects of this plant is available. In the order to find alternative bioactive compounds to control *Tirbolium castaneum*, the present study has screened the repellency and the efficacy of the crude ethanolic extract of *H. strobilaceum* as potential

botanical insecticide against this pest under laboratory conditions. Te effect of this extract on the detoxification enzyme Glutathione S-transferase (GST) and the neuroenzyme Acethylcolinesterase (AChE) was also investigated.

MATERIAL AND METHODS

Plant collection and preparation of crude ethanolic extract

The plant *H. strobilaceum*was collected from Sidi Mehdi, Touggourt region (Southeastern part of Algeria) during autumn season in 2015. The plant was taxonomical identified and confirmed by Dr Benhouhou from the National High College of Agriculture, Algiers, Algeria. Aerial parts of *H. strobilaceum* were air dried in the shade and grounded into fine powder using electrical blender. The powder was stored at room temperature in hermetically sealed plastic boxes until extraction.

Insect rearing

Initial stock culture of *T. castaneum* was obtained from entomology laboratory of National Institute of Plant Protection, El-Harrach, Algiers. Beetles were reared, at zoology laboratory of Boumerdes University, in glass containers (0.5 L) containing wheat flour mixed with brewer's yeast (10:1; w/w). The culture was maintained in the dark in growth incubators at 28-30°C and 70-80 % RH. Adults of 1-5 days post emergence were used in experiments.

Preparation of crude ethanolic extract and phytochemical screening

The crude ethanolic extract of the aerials parts of *H. strobilaceum* was prepared by macerating the powder for 3 days in ethanol, followed by filtration and evaporation at 40°C. The dried extract was kept at 4 °C until further use. The ethanolic extract was tested for plant secondary metabolites, alkaloids, phenolic compounds, flavonoids, saponins, tannins, iridois and coumarins. Phytochemical screening of the extract was carried out according to the standard method of Dohou et al. (2003). Visible color change or precipitate formation was taken into consideration for presence (+) or absence (-) of particular active constituents.

Repellent activity

To assess the repellency activity of *H. strobilaceum* crude ethanolic extract against *T. castaneum* adults, an area preference method of McDonald et al. (1970), with sligh modifications, was adopted. The test was carried out under the same conditions described above for the mass rearing using glass Petri dishes as containers. Filter paper (Whatman N° 1, 9 mm) was cut in half.

The test extract was tested at the concentration of 1000 µg. Test compounds were dissolved on acetone and 500 µl of the test solution were applied uniformly to half filter paper disc. Another half was treated with acetone only.

Treated and untreated halves were air dried, carefully fixed and placed in Petri dishes. For each test twenty adults insects (1-5 days post emergence) were introduced at the centre for the Petri dishes. The number of insects on the two halves disks were recorded after 2 and 4h from the beginning of the test. The percentage of repellency was calculated as follows:

$$PR\ (\%) = (Nc-Nt)/\ (Nc+Nt)\ x\ 100$$

Nc: Number of insects on control part
Nt : Number of insects on treated part

The average values were then categorized according to the following scale:

Class	Repellency rate (%)
0	>0.0l to 0.1
I	0.1 to 20
II	20.1 to 40
III	40.1 to 60
IV	60.1 to 80
V	80.1 to 100

Contact toxicity test

The bioassay was carried out using five concentrations of the crude ethanolic extract: 100, 200, 400, 800 and 1000 µg/insect. Test solutions were prepared using acetone as solvent. Unsexed adults insects were immobilized 15 min before the beginning of the test. Aliquots of 5 µL of each tested concentration were topically applied onto the thorax of insects using micropipette applicator. For each concentration twenty insects were used in 5 replicates. Acetone was used for the control test. After treatment, insects were transferred into glass Petri dishes containing a mixture of wheat flour and brewer's yeast given as food. All treated and control insects were kept under the same conditions as described for the insect rearing. Insect's mortality was recorded daily and LD_{50} was calculated.

Acetylcholinesterase and Gluthatione S-transferase assays

The AChE activity was carried out following the method of Ellman et al. (1961) using acetylthiocholine as a substrate. Adults of *T. castaneum* were sampled from control and treated groups (1000 µg/insect). Pools of twenty adults were homogenized in the solution containing 38.03 mg of ethylene glycol tetraacetic (EGTA), 1mL Triton X-100, 5.845 g NaCl and 80 mL Tris buffer (10Mm, pH 7). The homogenate was centrifuged (5000 g for 5 min at 4°C), and the resulting supernatant was used for enzymatic assay. The AChE activity was measured in aliquots (100 µL) of resulting supernatants added to 100 µL of 5-5' dithiobis-(2-nitrobenzoic acid) (DNTB) in Tris buffer (0.01 M, pH 8) and 1 mL Tris (0.1 M, pH 8). After 5 min, 100 µL of acetylthiocholine was added. Measurements were conducted at a wavelength of 412 nm with a run time of 20 minutes.

GST activities were determined with the soluble fraction as enzyme source. GST activities toward 1-chloro-2, 4-dinitrobenzene (CDNB) were measured according to Habig et al. (1974). Treated (1000 µg/insect) and control insect were homogenized in sodium phosphate buffer (0.1 M, pH 6) and centrifuged (14000 g, 30 min). Two hundred microliter of the resulting supernatant was added to 1.2 mL of reaction mixture containing 1Mm of CDNB

and 5 Mm of reduced glutathione (GST) in the homogenization buffer. Changes in absorbance were recorded at 340 nm. Total protein content was determined according to method of Bradford (1976) using bovine serum albumin as a standard. Enzyme activities were expressed as $nMmin^{-1}mg^{-1}$ proteins. The percentage of activation or inhibition was calculated for each enzyme.

Statistical analysis

Results are expressed as means ± standard deviation (SD). To identify significant effects of the treatments on the variables measured, data were submitted to a monofactorial ANOVA using XLSTAT 7.5.2. Means were compared using Tukey's HSD test ($P< 0.05$).

RESULTS AND DISCUSSION

Phytochemical screening

The crude ethanolic extract of *H. strobilaceum*was subjected to qualitative phytochemical screening to identify presence or absence of selected chemical constituents using classical methods of analysis. From phytochemical screening (Table1) we observed that the studied plant contains different groups of secondary products. The plant is rich in flavonoids, tannins, alkaloids, coumarins, antocyans and saponins. The study also shows that this plant does not contain iridoids.

Table 1.Qualitative phytochemical screening of crude ethanolic*H. strobilaceum.*

Alkaloids	Antocyans	Comarins	Tannins	Saponins	Iridoids	Flavonoids
+	+++	++	++	+++	-	+++

(-): Absent ; (+): Low presence ; (++): Moderate presence ; (+++): Strong presence

Contact toxicity

The tested extract exhibited obvious toxicity against adults of *T. castaneum* (Fig. 1). Indeed, the five tested doses caused mortalities of 15, 33.3, 41.6, 48.3 and 70%, respectively, after 6 hours of exposure. The highest dose (1000 µg/insect) generated 100 deaths after 96 h of exposure. The LD_{50} calculated 24 h after treatment was 225.4 µg/insect.

Repellent effect

The extract tested at dose of 1000 µg/insecte has a good repellent effect on adults of *T. castaneum* (Table 2). Repellent action varied depending on the time exposure. Indeed, the repulsively rate calculated after two hours of exposure was 60%. After 4 h of exposure, the PR value was 89.26 ±5.2 %.

Table 2.Repellent activity of the crude ethanolic extract of the plant *H. strobilaceum* against adults of *T. castaneum* at differents exposure times.

Exposure time	2 h	4 h
% of repellency	60 ± 02^{b}	89.26 ± 5.2^{a}
Class	III	V

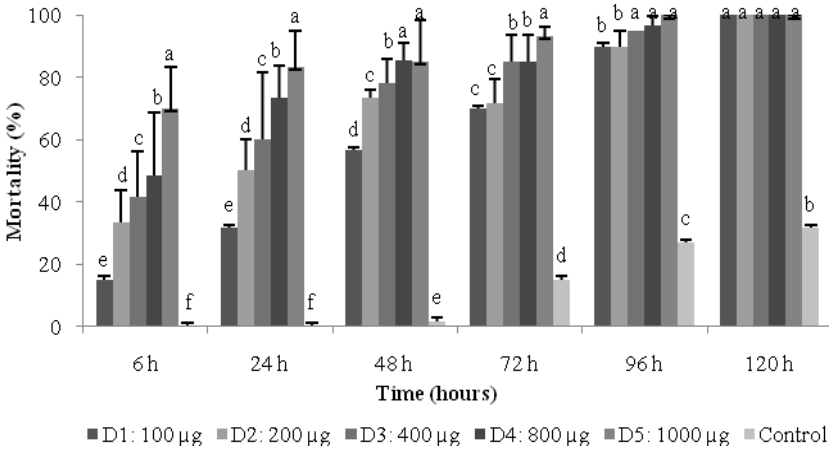

Figure 1. Toxicity of the crude ethanolic extract of *H. strobilaceum* applied topically to the adults of *T. castaneum* (Mean ± SD). N = 20 insects/replicate. Values followed by the same letter are not significantly different at P < 0.05according to Tukey's test.

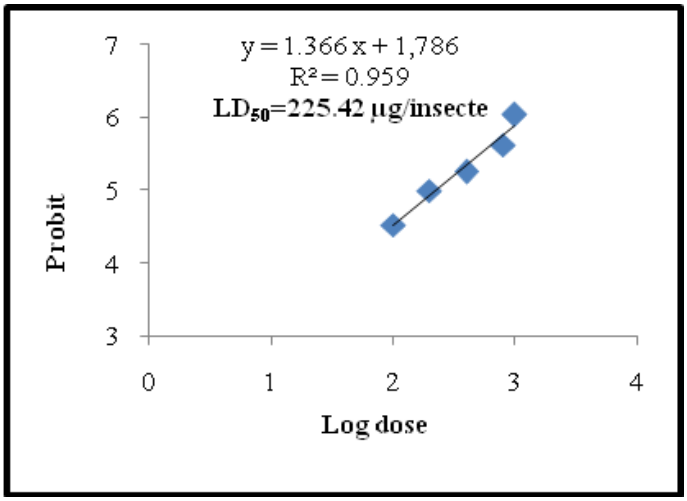

Figure 2. Effect of crude ethanolic extract of *H. strobilaceum* applied topically on the adults of the red flour beetle *T.castaneum* noted after 6 h of exposure (Mean ± SD). N = 20 insects/replicate.

Enzymatic assays

For enzymatic activities (Figs. 3 and 4), the results showed that the crude ethanolic extract of *H. strobilaceum*, applied at the dose of 1000 µg/insect inhibited significantly the AChE activity.

However, the extract has no significant effect on the GST activity. Indeed, the values of the enzyme activity are comparable between the control and treated series.

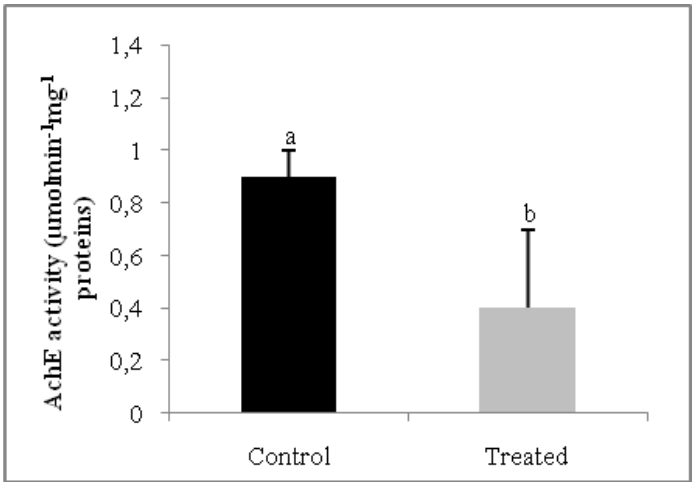

Figure 3. Effect of crude ethanolic extract of *H. strobilaceum*on AChE activity of the adults of the red flour beetle *T. castaneum* (Maen ± SD), N=20 insects. Different letters denote significant differences (Tukey's test, $p < 0.05$).

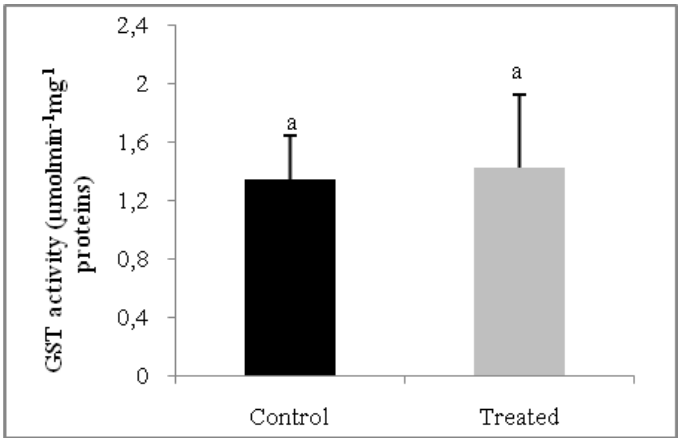

Figure 4. Effect of crude ethanolic extract of *H. strobilaceum*on GST activity of the adults of the red flour beetle *T. castaneum* (Maen ± SD), N=20 insects. Different letters denote significant differences (Tukey's test, $p < 0.05$).

Plant secondary compounds are an important biochemical basis for the plant defense against herbivores insects. Those compounds such as phenolics, alkaloids and proteins amino-acids are deleterious to insects and other herbivores by divers ways. Thus, they play a key role in plant defensive response to pests through acute toxicity and enzyme inhibition (Zhang et al. 2013). Phytochemical study of the studied plant showed the presence of various groups of natural products. The plant is rich in flavonoids, tannins, alkaloids and coumarins. The study also shows that this plant does not contain iridoids. The phytochemistry of this halophyte remains very poorly known, only two studies indicate the isolation

of bio-actives compounds from this plant. Four coumarins: coumarin, hydroxy-3-methylcoumarin, oreoselone, and heraclenin where isolated from the aerial part of *H. strobilaceum* (Miftakhova et al. 2001). Also, a new n-alkyl ester of 3,4-dihydroxycinnamic acid (caffeic acid) has been isolated from this plant by Gibbons et al. (1999). Aerial plant parts contain 6.9% protein, 2.15% fat, 17% fiber and 40.1% ash (El Shaer et al. 1991).

On insecticidal level, positive results for contact and repellent activity of the tested extract were obtained against the adults of the red flour beetles. Indeed, it was very clear that the percentage of mortality was directly proportional to the concentration of the test extract. Mortality increased from higher to lower concentration. The five tested doses caused mortalities of 15, 33.3, 41.6, 48.3 and 70 %, respectively, after 6 hours of exposure. The highest dose (1000 µg/insect) generated 100 deaths after 96 h of exposure. The LD_{50} calculated 24 h after treatment was 225.4 µg/insect. The insecticidal properties of the crude extract of this plant could be rationalized by synergistic action of its all compounds, major compounds and some other minor compounds. Many plant extracts and essential oils have been reported to be effective against pests in stored products. The study conducted by Zardi-Bergaoui et al. (2008) indicate that the ethyl acetate crude extract and eight fractions A_3, A_4, P_8, P_{10}, F_2, F_3 and F_7 of *Anacyclus cyrtolepidoides* showed a significant inhibitory effect of *T. confusum* growth. 100 % mortality of adults was achieved 12 days after treatment using fractions A_4, P_8 and F_7. In a recent study of Phankaen et al. (2017), a significant mortality was recorded on fumigation assay with the dichloromethane extract of *Coffea arabica*. However, the active ingredient isolated of this extract did not induce similar toxicity as the dichloromethane extract. Results obtained by Saidana et al. (2010) indicated that the methanolic extract of tunisian halophyte *Tamarix boveana* caused significant and early mortalities and growth inhibition of the insect *Trogoderma granarium* at the concentration of of 50 µg/disc.

In the present study, the plant extract showed potent repellent effect on *T. castaneum* adults. The PR value was 89.26 ±5.2 % after 4 h of exposure at the testing concentration (1000 µg/insect). Many researchers have reported on the repellency of essential oils and plant extracts against insects, especially those infesting stored products. Essentials oils extracted from *Stureja* spp were strongly repellent against *T. castaneum* adults at the concentration of 1% (v/v) after 4 hours of exposure (Taban et al., 2017). Phankaen et al. (2017) obtained a very strong repellency with the active compounds isolated from *C. Arabica* against *T. castaneum* adults.

Plant secondary compounds such as phenolics, alkaloids and non-protein amino acids are deleterious to insects and other herbivores in diverse ways. Thus, they play a key role in plant defensive response to pests through acute toxicity and enzyme inhibition (Zhanget al., 2013). The understanding of the mechanism of action of the extract of our plant on certain target enzymes remains important for a possible formulation of a bio-insecticide. The measurement of the activity of the GST and the AChE was carried out in this perspective. For enzymatic

activities, data showed that the crude ethanolic extract of *H. strobilaceum*, at the dose of 1000 µg/insect, has no significant effect on the GST activity. However, the extract inhibited the AChE activity. Our results were in agreement with those of Mami-Maazoun et al. (2017) wich showed that *Urginea maritima* bulbs extract was able to interfere with *Sitophylus oryzae* acetylcholinesterase enzyme andexhibited significant inhibitory effect on acetylcholinesterase activity. This inhibition could possibly be due to its high content in phenolic compounds and alkaloids. Once they penetrated inside the insect body, alkaloids and phenolic compounds reached nervous system and inhibited activity of acetylcholinesterase.

CONCLUSION

Based on the present study, it can be concluded that the crude ethanolic extract of the Saharian halophyte plant *H. strobilaceum* tested for its repellent activity and contact toxicity, exhibited obvious effects against the red flour beetle *T. castaneum*. For its acethylcolinesterase inhibition, this extract can be used as an insecticide against the pests of stored products. However, further tests are needed to develop a formulation of this natural insecticide.

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Katarzyna TAJCHMAN,
Marek BOGDASZEWSKI, Edyta KOWALCZUK-VASILEV,
Żaneta STEINER-BOGDASZEWSKA, Paweł BOGDASZEWSKI [1]

MINERAL CONCENTRATIONS IN THE PLASMA OF YOUNG FARMED FALLOW DEER (*Dama dama*) IN RELATION TO THE FEEDING SYSTEM

SUMMARY

Ruminant breeders, who strive to feed their animals properly, focus their attention on three components of diet: metabolic energy, total protein and raw fibre. Properly balanced nutrition should take into account another element, i.e. the proper level of mineral-vitamin compounds.

The paper shows the effects of nutrition on the content of micro- and macro-elements in the blood of farmed fallow deer reared in two systems. Male calves constituted the main study group. All the animals had constant access to water and hay. One of the groups of the animals kept in a special rearing house received a complementary mineral feed mixture for calves "Cielak plus 2.5%" produced by LNB (Cargill, Poland); the administration followed manufacturer's recommendations. Additionally, the feed contained fodder composed of 70% of crushed oats, 15% of the "universal rapeseed concentrate" (producer: Eko-pasz, Mońki, Poland) containing 33% of crude protein, and 15% of the "universal soybean concentrate" (producer: Eko-pasz, Mońki, Poland) with 45% of crude protein. The second group was reared in a free-range system outside the calf-shed and was fed roughage feeds ad libitum.

The aim of this study was to determine the selected minerals in the plasma of young farmed fallow deer. The blood was collected at slaughter, which is a natural element in the farm rearing technology. Plasma P, Ca, Mg, Zn, Fe, and Cu levels were determined. Specific reference intervals are needed for each animal species for appropriate interpretation of haematological and serum biochemical results. Serum biochemical parameters, such as phosphorus, calcium, magnesium, zinc, copper and iron content, were determined using reagent kits (BioMaxima, Lublin, Poland), according to manufacturer's protocol and a random access biochemical analyser Metrolab 2300 GL (Metrolab SA, Buenos Aires, Argentine). The preliminary results confirm the beneficial effect

[1]Katarzyna Tajchman (corresponding author: katarzyna.tajchman@up.lublin.pl), Department of Ethology and Animal Welfare, University of Life Sciences in Lublin, POLAND; Marek Bogdaszewski, Żaneta Steiner-Bogdaszewska, Paweł Bogdaszewski, Institute of Parasitology PAN, Researche Station in Kosewo Górne, POLAND; Edyta Kowalczuk-Vasilev, Institute of Animal Nutrition and Bromatology, University of Life Sciences in Lublin, POLAND
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of feeding young animals diets containing higher protein contents and mineral mixes.

Keywords: *Dama dama*, nutrition, micro- and macro-elements.

INTRODUCTION

In Poland, Cervidae breeding was made possible by virtue of the Act of 29 June 2007 on organisation of breeding and reproduction of farmed animals (Journal of Laws 2007 no. 133, item 921). As specified in chapter 1, Art. 2, Par. 1 c), Cervidae species such as the red deer (*Cervus elaphus),* sika deer (*Cervus nippon*), and fallow deer (*Dama dama*) reared in farm conditions for production of meat and pelts are classified as livestock animals if they originate from closed or free-range rearing and breeding systems. In other countries, other cervid rearing and breeding objectives are additionally implemented, i.e. panty production (antlers in a growth form) and the use of animals in tourism and agritourism (Janiszewski *et al.*, 2014, Dmuchowski 2003).

To provide their animals with proper nutrition, breeders focus on three nutritional components of feed, i.e. metabolic energy, total protein, and raw fibre. Properly balanced diets should provide an appropriate level of mineral-vitamin compounds as well. Cervidae, including the fallow deer, are characterised by special nutritional requirements and are particularly susceptible to mineral compound deficiencies due to the increased demand for minerals during the period of development and ossification of antlers. This is a special time during the rearing period, as osteoporosis of the skeletal system may then develop, leading to weakening of the organism.

In Cervidae, the quality of the first antler is an indicator of the ontogenetic quality and a phenotypic trait that can reflect the quality of food (Gaspar-Lopez *et al.*, 2008). In the farm breeding practice, there is a possibility and, hence, a necessity to offer animals appropriate living conditions directly after birth and to provide properly balanced nutrition to hinds and calves immediately after weaning.

MATERIAL AND METHODS

The research was carried out at the Research Station of the Institute of Parasitology, Polish Academy of Sciences, Kosewo Górne, Poland, in 2014-2016. Eighteen male calves constituted the main study group. All animals had constant ad libitum access to water and hay. The fallow deer were divided into two groups of equal size. One group was kept in a special rearing building and the other one was reared in a free-range system.

Each animal received 260 g of a mixture per day with the following composition: 70% of crushed oats in 15% of “universal rapeseed concentrate” (producer: Eko-pasz, Mońki, Poland) containing 33% of crude protein, and in 15% of “universal soybean concentrate” (producer: Eko-pasz, Mońki, Poland) with 45% crude protein content. Additionally, the diet was supplemented with a complementary mineral feed mixture for calves “Cielak plus 2,5%” (produced by LNB, Cargill, Polska) in accordance with manufacturer’s recommendations. Our

unpublished observations revealed homogeneous feed intake by the animals, although the exact level of feed intake by each animal was not determined.

The effects of intensive nutrition with the use of the diet described above on the blood levels of micro- and macro-elements in the farmed fallow deer reared in two different systems were analysed. The aim of this study was to determine selected minerals in plasma in young farmed fallow deer. Blood was collected at slaughter in the second year of animals' age (August 2016). Plasma P, Ca, Mg, Zn, Fe, and Cu levels were determined. Specific reference intervals are needed for each animal species for appropriate interpretation of haematological and serum biochemical results. Serum biochemical parameters, such as phosphorus, calcium, magnesium, zinc, copper and iron content, were determined using reagent kits (BioMaxima, Lublin, Poland) according to the manufacturer's protocol and a random access biochemical analyser Metrolab 2300 GL (Metrolab SA, Buenos Aires, Argentine).

The animal body weight was measured at the beginning (November 2014) and end (May 2015) of the study period and in August 2016. The measurements were carried out with the use of a set of MP 800 sensors coupled with a Tru-test DR 3000 weight reader. As declared by the manufacturer, the accuracy of this set is +/- 1% and the minimum resolution is 0,1 kg. To obtain additional information about the ontogeny of the young fallow deer males, the growing antlers with the pedicle (at the skull base) were measured twice. The measurements were done at the age of 11 and 26 months using a tape measure and the mean values of the measurement of both beams were noted (in each case, the differences in the length did not exceed 1 cm).

All the data were analysed with Statistica software ver. 10 (StatSoft, Tulsa, OK, US, 2011). The normality was assessed using the Kolmogorov-Smirnov test, and Levene's homogeneity of variance test was applied to examine the equality of variances. The data obtained were analysed with the ANOVA method using one-way analysis of variance and calculating the mean values for the treatments, standard deviation (SD), and the standard error of the mean (SEM). The means were compared using Tukey's test. All statements of significance were based on a probability $P \leq 0.05$ and P values between 0.05 and 0.1 were considered as a trend.

RESULTS AND DISCUSSION

A positive effect of the mineral supplementation before the second year of age of the fallow deer was observed. Animals receiving the supplemented diet exhibited increased plasma levels of the analysed minerals (Zn, P, Mg, Cu, Ca, Fe). Higher contents of some mineral compounds were found in the animals kept in the calf-shed. The values were higher on average by 5,46 umol/l, 2,82 umol/l, and 0,23 mmol/l in the case of iron, zinc, and phosphorus, respectively. The levels of magnesium, calcium, and copper did not differ substantially and were slightly higher in animals receiving the additional supplementation. Significant statistical differences in the level of calcium were noted between the groups. Its mean content in the control group was 2,3 mmol/l +0,12, and a higher value, i.e.

2,42 mmol/l +0,12, was determined in the experimental group, which indicates a high demand of fallow deer for calcium as well as magnesium, phosphorus, and copper during the development and mineralisation of antlers (Table 1, Table 2).

Table1. Comparison of content of selected micro- and macro elements (Zn, P, Mg, Cu, Ca, Fe) in fallow deer plasma.

	No. animal	Zn umol/l	P mmol/l	Mg mmol/l	Cu umol/l	Ca mmol/l	Fe umol/l
control group	1	9,60	2,20	0,85	13,80	2,47	28,34
	2	8,26	1,67	0,78	14,94	2,18	21,52
	3	6,19	1,71	0,74	15,61	2,23	16,22
	4	7,74	1,52	0,80	13,45	2,35	19,66
	5	9,06	1,50	0,81	15,70	2,42	45,03
	6	6,34	1,69	0,80	14,81	2,21	22,30
	7	9,97	2,33	0,87	15,10	2,45	26,10
	8	8,20	1,77	0,69	14,44	2,19	27,20
	9	8,35	1,65	0,87	13,34	2,22	26,60
average		**8,19**	**1,78**	**0,80**	**14,57**	**2,30**	**25,88**
experimental group	10	6,58	1,86	0,98	13,01	2,40	32,67
	11	8,19	2,16	0,93	16,10	2,40	34,59
	12	11,04	1,77	0,85	15,63	2,45	30,75
	13	5,95	2,16	0,82	12,87	2,28	27,02
	14	15,99	1,62	0,73	14,70	2,39	17,20
	15	17,84	2,37	0,90	13,71	2,70	33,02
	16	14,68	2,19	0,82	18,56	2,43	46,21
	17	11,28	1,90	0,80	14,75	2,32	35,60
	18	7,53	2,05	0,88	14,20	2,44	25,02
average		**11,01**	**2,01**	**0,86**	**14,84**	**2,42**	**31,34**

Besides the higher levels of the minerals in the plasma of the farmed fallow deer, a positive effect of the mineral supplementation on the growth of the first antlers was shown. The antlers were on average by ca. 2 cm longer in the animals kept in the calf-shed during the first measurements and by 3,6 cm longer in the subsequent year. Additionally, more uniform growth of antlers was observed in the animals receiving the supplemented diet.

Table 2. Analysis of the variation of micro- and macro element content in plasma between groups with standard deviation of probability at the level P≤0,05.

Group	control *mean ± SD*	experimental *mean ± SD*	P value	SEM
Zn umol/l	8.19 ± 1.30	11.00 ± 4.33	0.080	*0.808*
P mmol/l	1.78 ± 0.29	2.01 ± 0.24	0.088	*0.066*
Mg mmol/l	0.80 ± 0.06	0.86 ± 0.08	0.101	*0.017*
Cu umol/l	14.58 ± 0.88	14.84 ± 1.77	0.698	*0.321*
Ca mmol/l	2.30^{b} ± 0.12	2.42^{a} ± 0.12	0.046	*0.031*
Fe umol/l	25.88 ± 8.21	31.34 ± 8.01	0.173	*1.969*

During the investigations, the animals were weighed. The fallow deer from the experimental group exhibited higher body weight (by approx. 4,5 kg) at the age of 11 months and weighed on average ca. 32,3 kg. In turn, the control animals weighed on average 30,2 kg. After 26 months, more equal body weight gains were noted in both groups, which may be associated with the identical diets provided to the animals in the two groups in the second year. The mean body weight of the animals from the two groups differed only by 0,3 kg in that period. The animals from the group fed with the supplemented diet reached a body weight of 42,6 kg (Table 3).

The analysis of changes in the biometric parameters revealed a significant difference between the control group and the group of animals fed with the supplemented diet. After 11 months, the mean body weight of the animals from the former group was 28,5 kg +2,38, while the animals from the latter group were characterised by higher values, i.e. 32,3 kg + 4,08 (Table 4). Significant correlations between the groups were shown in the animal body weight gain after 26 months. The groups differed between each other, i.e. the body weight gain was 18,1 kg + 2.88 in the control group and 14,9 kg + 2,77 in the experimental group. There were also significant correlations in terms of the antler growth values between the groups in both measurement periods. The mean antler length after 11 months was 2,5 cm + 0,81 in the control group and 4,5 cm + 2,28 in the experimental group. After 26 months, the mean antler length was 9,7 kg + 2,26, whereas a higher value, i.e. 13,3 kg + 2,99, was noted in the experimental group (Table 4).

The results of this study are comparable to those presented in the literature. In their investigations of the red deer, Peinado et al. (1999) reported a plasma calcium concentration of 2,24 mmol/l and an elevated level of phosphorus, i.e. 3,87 mmol/l. The concentrations of calcium, phosphorus, and magnesium determined by Kuba (2014) (mean values: Ca- 1,96 mmol/l, P- 1,83 mmol/l, Mg- 0,6 mol/l) were lower than the values in the non-supplemented fallow deer kept in the free-range system (Ca- 2,30 mmol/l, P-1,76 mmol/l, Mg- 0,8 mmol/l). This may be associated with the better quality of the standard diets provided in the farm where the present study was carried out.

Table 3. Body weight and length of antler at fallow deer in the sixth, eleventh, and 26th month of life.

	No. animal	initial body weight (kg) (animals 6-month, winter)	final body weight (kg) (animals 11-month, spring)	Increase body weight (kg)	Average length antlers (cm) (animals 11-month, spring)	mass of 26 months old animals (kg) summer	Increase body weight (kg) summer	Average length antlers after 26 months (cm) summer	Increase antlers (cm)
control group	1	30,5	31	0,5	2,2	45,0	14,5	10,7	8,5
	2	29,5	32,5	3	1	41,5	12	5,9	4,9
	3	26,5	28	1,5	2	41,5	15	10	8
	4	24	27	3	3,5	39,5	15,5	11,3	7,8
	5	27,5	31,5	4	2,9	46,0	18,5	12,3	9,4
	6	26	28	2	2	40,5	14,5	6,1	4,1
	7	27,5	32,5	5	2,7	45	17,5	10,8	8,1
	8	28	29,5	1,5	3,5	43,5	15,5	11	7,5
	9	30	32	2	3	38	8	9,4	6,4
average		27,7	30,2	2,5	2,5	42,3	14,6	9,7	7,2
experimental group	10	30	33,5	3,5	6,5	39,0	9,0	11,8	5,3
	11	30	33,5	3,5	5,5	44,5	14,5	14,9	9,4
	12	22,5	28	5,5	3,5	38,0	15,5	12,8	9,3
	13	30,5	36	5,5	8	49,0	18,5	19,0	11
	14	25	31	6	3	43,0	18,0	8,3	5,3
	15	21,5	24	2,5	1	36,0	14,5	11,3	10,3
	16	32,5	34,5	2	5	47,0	14,5	15,3	10,3
	17	30,5	37	6,5	6	46,5	16,0	13,2	7,2
	18	27,5	33	5,5	2	41,0	13,5	12,3	10,3
average		27,7	32,3	4,5	4,5	42,6	14,9	13,3	8,8

Table 4. Analysis of body weight variance and antler length between fallow deer groups with a standard deviation of probability at the P≤0,05.

Group	control *mean ± SD*	experimental *mean ± SD*	P value	SEM
Initial body weight (kg) (animals 6-month)	27.72 ± 2.08	27.77 ± 3.91	0.970	*0,715*
Final body weight (kg) (animals 11-month)	30.22 ± 2.14	32.28 ± 4.08	0.199	*0,785*
Increase body weight (kg)	2.50^{b} ± 1.39	4.50^{a} ± 1.64	0.013	*0,424*
Average length antlers (cm) (animals 11-month)	2.5^{b} ± 0.81	4.5^{a} ± 2.28	0.027	*0,458*
mass of 26 months old animals (kg)	42.28 ± 2.75	42.67 ± 4.47	0.827	*0,849*
Increase body weight (kg)	14.56 ± 3.08	14.89 ± 2.77	0.812	*0,671*
Average length antlers after 26 months (cm)	9.7^{b} ± 2.26	13.3^{a} ± 2.99	0.013	*0,739*
Increase antlers (cm)	7.2 ± 1.73	8.8 ± 2.21	0.124	*0,490*

Fallow deer plasma was sampled in summer, a year after the supplementation. In this period, animals should not suffer from nutritional deficiencies due to the good quality of pastures. Yet, relatively large differences in the plasma content of micro- and macroelements were observed between the supplemented and non-supplemented animal groups. This indicates a low level of compensation of nutritional deficiencies arising at a younger age, in particular during the weaning period, when animals should receive special care. The composition of the hinds' milk itself has a significant effect on Cervidae. It has been shown in the Iberian red deer (Cervus elaphus hispanicus) that the composition of milk exerts an impact on the morphological traits of the first antler, in particular its length. The length of the antler was measured from the value of 2,5 cm to the end of its growth. Between weeks 2 and 8, the gain was inconsiderable, i.e. from 0,5-3,0 cm to 1-19,0 cm. The greatest increase in the length, i.e. 10,0-55,0 cm, was noted between week 8 and 20. In the final period of growth between week 20 and 28, the antler length was 21,5-55,5 cm. In comparison with wild-living animals in which the length of the first antler can be highly variable in some individuals and range from 15 cm to 60 cm, it can be observed that the value is stable in most individuals provided with adequate nutrition and reaches on average 38,3 cm (Gaspar-Lopez et al., 2008). This thesis is also confirmed by the results of the present study carried out on farmed fallow deer.

The issue of appropriate feeding is especially important in Cervidae calves, whose survival of the first winter depends on proper body weight (Fenessy et al., 1991). Studies have shown that early growth in young animals, especially in males, is highly important, as it determines some antler traits (weight and length) in adult deer and influences future reproductive success (Gómez et al., 2006, 2008, Landete-Castillejos et al., 2007 a).

In favourable environmental conditions ensuring good health status of animals, the young red deer can begin puberty up to two months earlier (at the age of 14-16 months) (Asher and Cox, 2013). On the other hand, adverse conditions can considerably delay this moment even until the third or fourth autumn after birth. This boundary is unstable and is reached when animals achieve 65-70% of the target weight, which is an effect of the availability and quality of feed (Asher et al., 2011, Cilulko, 2011).

Most studies on cervid nutrition conducted so far have mainly been focused on the content and quality of proteins and energy compounds. However, the role of micro- and macroelements, in particular calcium, phosphorus, and magnesium, is highly important. These mineral compounds build up the antler bone mass in Cervidae (Landete-Castillejos et al., 2007 b). Special attention should be paid to the high demand of Cervidae for calcium, which is supplied with feed only in 25-40%, while the other amount originates from the pool deposited in their bones (Muir et al., 1987).

The effect of supplementation of diets in farmed Iberian red deer (Cervus elaphus hispanicus) has been investigated. For three years post weaning, the animals were administered higher amounts of mineral compounds, i.e. Ca, P, Mg, K, Na, S, Cu, Fe, Mn, Se, Zn, B, and Sr, in a complementary diet. The higher availability of the micronutrients in the feed was reflected in the chemical composition and mechanical properties of bones. A significant difference in biometric parameters was shown between the control group and animals receiving the supplemented diet. The mean body weight of animals from the former group was 83,6 kg +1.6; in the latter group, the value was higher, i.e. 90,1 kg + 1,4. Similarly, the KFI value differed between the groups and was 65,42 kg + 9,9% and 131,26 kg + 13,7%, respectively. No differences in the chest circumference, body length, femur length, cortical thickness, or mechanical properties were found (Olguin et al., 2013).

CONCLUSIONS

The results have demonstrated a beneficial effect of mineral supplementation on the weight and form of antlers and the ontogenic quality of the animals. A significant correlation was observed in the case of calcium, whose plasma content was higher in the experimental group and which appeared to be the most vital macroelement for the structure of antlers. The other micro- and macroelements (Zn, P, Mg, Cu) also play an important role in farmed fallow deer. Their plasma content was also higher in the supplemented group. Therefore, special attention should be placed on mineral-vitamin supplementation during the

calf wintering period, as it influences the subsequent years of animal life and contributes to achievement of appropriate weight and more uniform growth of antlers.

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Galina SOKOLENKO, Irina PONOMAREVA [1]

THE USE OF JERUSALEM ARTICHOKE FOR OBTAINING THE YEAST BIOMASS FOR FOOD AND FEED PURPOSES

SUMMARY

Researches on the use of Jerusalem artichoke tubers in the production of yeast biomass for food and feed purposes have been conducted. It was revealed that inulinase activity of yeast strains of *S. cerevisiae G* and *K. marxianus VKM Y*-1148 was 590 u / g and 150 u / g respectively at the cultivation in the inulin-containing medium. Strain *S. cerevisiae G* grows well in non-hydrolyzed juice of the Jerusalem artichoke obtained from fresh tubers. The juice of the Jerusalem artichoke has a high content of inulin (18.7%), phosphorus, potassium, B vitamins, biotin and can be used as a culture medium of yeast without mineral salts. The yield of biomass by culturing the yeast with aeration (30 °C, 24 h) was 60 g / L. The addition of $(NH_4)_2SO_4$ into the Jerusalem artichoke juice (0.6%) increased the accumulation of yeast biomass up to 74.6 g /L which is 24% higher of yeast biomass grown in beet molasses medium. The stimulating effect of the Jerusalem artichoke juice on *K. marxianus Y*-1148 during milk whey fermentation (30 ºC, with aeration) has been established. The maximum stimulating effect (2.8 times) is shown at the 15% content of Jerusalem artichoke in milk whey and during 24 h of incubation. The use of Jerusalem artichoke increases the yield of yeast biomass and enriches it with probiotic substances. It allows replacing the traditional molasses by the Jerusalem artichoke and to reduce the cost of production by increasing the yield of yeast biomass and by excluding mineral salts.

Keywords: *Helianthus tuberosus* L., inulin, yeast, *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*.

INTRODUCTION

At present, highly effective methods of processing and utilization of plant raw materials are of great importance for solving food, energy and environmental problems. In this respect, an inulin-containing raw material, in particular Jerusalem artichoke (*Helianthus tuberosus* L.), is of great practical interest. The value of this plant is due to high yield, high-grade chemical composition, and ecological plasticity. Tubers Jerusalem artichoke is a rich source of carbohydrates, primarily fructosans, a significant part of which is inulin (73-86%). Inulin is a linear biopolymer consisting of fructose residues bound by β-1,2 bonds. At complete hydrolysis of inulin exposed inulinase (2,l-β-D-

[1]Galina Sokolenko (corresponding author: galigri@mail.ru), Irina Ponomareva, Voronezh State Agrarian University named after Emperor Peter the Great, RUSSIA

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fructanfructanohydrolase) produced 95% fructose, 5% glucose, which are utilized by microorganisms (Yu et al., 2010). The tubers of Jerusalem artichoke contain significant amounts of macro- and microelements, nitrogenous substances, vitamins, including biotin, necessary for the development of microorganisms. Therefore Jerusalem artichoke can be considered as an alternative source of carbohydrates for biotechnology productions.

Microorganisms of different taxonomic groups can synthesize inulinases: bacteria, actinomycetes, microscopic fungi, yeast (Chi Z. et al., 2009). Yeast *Kluyveromyces marxianus* are well-studied inulinase producers, and inulinases strains are used to produce fuel ethanol from inulin-containing raw materials, which allows to unite the processes of saccharification and fermentation of inulin (Yuan W et al., 2008a). Today there are a limited number of producer strains of inulinases among Saccharomyces cerevisiae, insufficient studies on the use of inulin-containing raw materials for the production of *S. cerevisiae* biomass.

In this connection, studies have been carried out on the application of Jerusalem artichoke tubers to obtain biomass of yeast *S. cerevisiae* and *K. marxianus*. These yeasts have GRAS status, are able to quickly accumulate biomass with high protein, which is balanced with amino acid composition. The biomass of these yeasts can be used for the production of the food ingredients and feed.

MATERIAL AND METHODS

The tubers of Jerusalem artichoke (*Helianthus tuberosus* L.) have been grown in the vicinity of Voronezh (Russia) and stored at 4-5 °C until use.

Microorganisms. As producers of biomass were used strain of baking yeast *Saccharomyces cerevisiae G* and a strain of *Kluyveromyces marxianus VKM Y*-1148. The inulinaseaktivity strain *Saccharomyces cerevisiae G* was obtained by the selection method in our laboratory (Sokolenko G.G., Karpechenko N.A., 2013; Sokolenko G.G., Karpechenko N.A., 2015), strain *Kluyveromyces marxianus* VKM Y-1148 was taken from the Russian Collection of Microorganisms (VKM).

Jerusalem artichoke juice was obtained by pressing from fresh crushed tuber of Jerusalem artichoke, heated diluted with water 2 times and heated at 100 degrees for 1 minute. After cooling, the juice was clarified by filtration and sterilized. The resulting Jerusalem artichoke juice contained sufficient quantities of nutrients necessary for yeast propagation (%): total nitrogen - 0.06; Reducing sugars - 0.8; Inulin - 18.72; Phosphorus - 0.046; Potassium - 0.3; Calcium - 0.022; Water - 89.32; pH is 6.0.

To determine inulinase activity of yeast, they were grown on a medium of the following composition (g / L): Inulin - 20.0; $(NH_4)_2SO_4$ - 5.0; KH_2PO_4 - 0.85- K_2HPO_4 - 0.15; $MgSO_4$ - 0.5; NaCl - 0.1; $CaCl_2$ - 0.1; Yeast extract – 2.0; pH is 5.0.

The culture medium based on molasses had a composition (g / L molasses diluted 4 times): $(NH_4)_2SO_4$ - 3.0; $(NH_4)_2HPO_4$ - 2.0; KCL - 1.0; pH is 5.0.

Fermentation was carried out in Erlenmeyer flasks (250 ml) with 50 ml of medium which was inoculated with overnight culture of yeast to a concentration of 1.0×10^7 cells/ ml and incubated at 30 °C on a rotary shaker (200 rpm). Samples were taken to determine the activity of inulinase and biomass.

K. marxianus was grown on unlighted curd whey, containing mineral salts (g / L whey): $(NH_4)_2SO_4$ - 3.0; $NH_4)_2$ HPO_4 - 2.0; KCL - 0.5. Fermentation was carried out with an initial concentration of inoculum of 1% at 30 ° C and 200 rpm. After fermentation the culture broth was heated 85-90 °C during 30 min to denature serum proteins and centrifuged at 6000g for 10 minutes to determine the mass yeast protein sediment. Was determined that yeast cells were 50% yeast protein.

The method for determining inulinase activity is based on the determination of reducing substances released during hydrolysis of the substrate under the action of the enzyme inulinase. One unit of inulinase activity is the amount of enzyme which catalyzes the formation of 1 g of reducing substance per 1 minute under the experimental conditions (Sokolenko G.G., Karpechenko N.A., 2013).

RESULTS AND DISCUSSION

Previously, we established the stimulating effect of inulin on the growth and reproduction of stains *S. cerevisiae* and *K. marxianus* when they have been grown in wort and whey (Sokolenko G.G. et al, 2010). In the present study it was revealed that inulinase activity of strains *S. cerevisiae G* and *K. marxianus Y*-1148 was 590 u / g and 150 u / g respectively at the cultivation in the inulin-containing medium (2% inulin). Strain *S. cerevisiae G* grows well in non-hydrolyzed juice of the Jerusalem artichoke obtained from fresh tubers. It makes it possible to use non-hydrolyzed Jerusalem artichoke juice as a growing medium for yeast. Known methods of growing *S. cerevisiae* using Jerusalem artichoke tubers presupposes hydrolysis of inulin with mineral acids, enzymatic preparations or activation of inulinases of Jerusalem artichoke (Patent of Russia No. 2064503, Patent of Russia No. 2144084, Patent of Russia No. 2301832, Patent of Russia No. 23590350). The studies on the cultivation of *S. cerevisiae G* on Jerusalem artichoke juice without preliminary hydrolysis of inulin have been carried out. It is established that the *S. cerevisiae G* grows well on the non-hydrolyzed juice of Jerusalem artichoke with the initial pH value (6.0) without the addition of mineral salts. The maximum accumulation of biomass was 60 g / L (cultivation for 24 h with aeration at 30 °C), which corresponds accumulation when grown on a molasses medium. Jerusalem artichoke has a low nitrogen content, and the introduction of $(NH_4)_2SO_4$ increased the accumulation of biomass. Research has been carried out on the effect of juice pH and concentration $(NH_4)_2SO_4$ on the yield of yeast biomass (Figure 1).

It was shown that the maximum accumulation of yeast biomass (74.6 g / L) was at pH 6.0 and concentration (NH_4) $_2SO_4$ - 0.6%, which is 24% more than with molasses (60 g / L) (Patent of Russia No. 2570628).

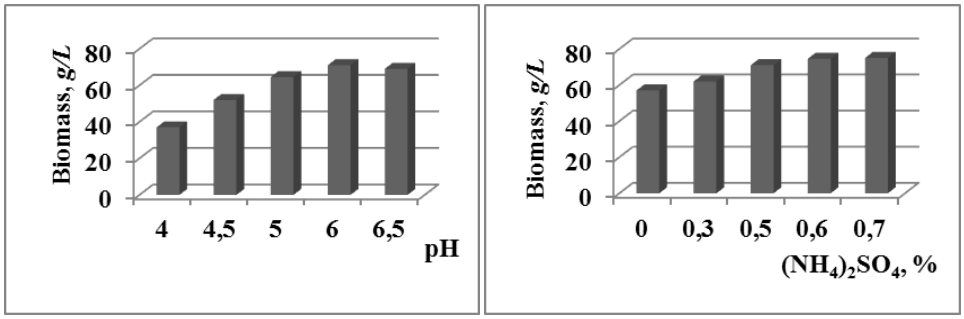

Figure 1. Effect of the pH of the Jerusalem artichoke juice and concentration $(NH_4)_2SO_4$ on the yield biomass of stain *S. cerevisiae G*.

The use of non-hydrolyzed Jerusalem artichoke juice allows replacing the traditional raw materials with the Jerusalem artichoke juice and reducing the production cost by increasing the yield of yeast biomass and excluding mineral salts.

Strain *K. marxianus VKM Y*-1148 synthesizes inulinase and β-galactosidase enzymes, is able to assimilate inulin and lactose in aerobic conditions and actively accumulate biomass (Fonseca GG et al, 2008). Therefore, it can be used in biotech production with using whey and inulin-containing plant material as substrates.

Studies have been conducted on the bioconversion of whey using *K. marxianus VKM Y*-1148 in yeast protein. Fermentation was carried out unlighted whey with aeration under batch cultivation conditions. It was revealed that as a result of the bioconversion in the whey the protein content increased 2.5 times, fat content increased 30 times, the product is similar to skim milk. The balance of essential amino acids was close to that of the "ideal protein". This allows us to recommend a yeast protein product for use for food and fodder purposes.

The juice of Jerusalem artichoke was added to the whey enriched with mineral salts, inoculated with an overnight yeast culture (1%), and fermented under aeration of the medium at 30 °C for 72 h.

It was revealed that the Jerusalem artichoke juice stimulates the growth and multiplication of yeast *K. marxianus VKM Y*-1148. The content in the serum of 15% of Jerusalem artichoke juice after 6 h of incubation increased the weight of yeast protein in comparison with the control in 2.4 times, after 8 h - in 2.5 times, after 24 h - in 2.8 times.

The influence of the concentration of Jerusalem artichoke juice and cultivation time on the efficiency of bioconversion of whey has been studied. Yeast was grown in whey with Jerusalem artichoke content 10%, 15%, 20% for 72 h, every 24 h in samples of culture liquid, the mass of the yeast protein precipitate was determined. The results of the studies are shown in Figure 2.

It is shown that during the fermentation of whey by *K. marxianus VKM Y*-1148 the Jerusalem artichoke juice stimulates the yeast and increases the yield of the bioconversion product.

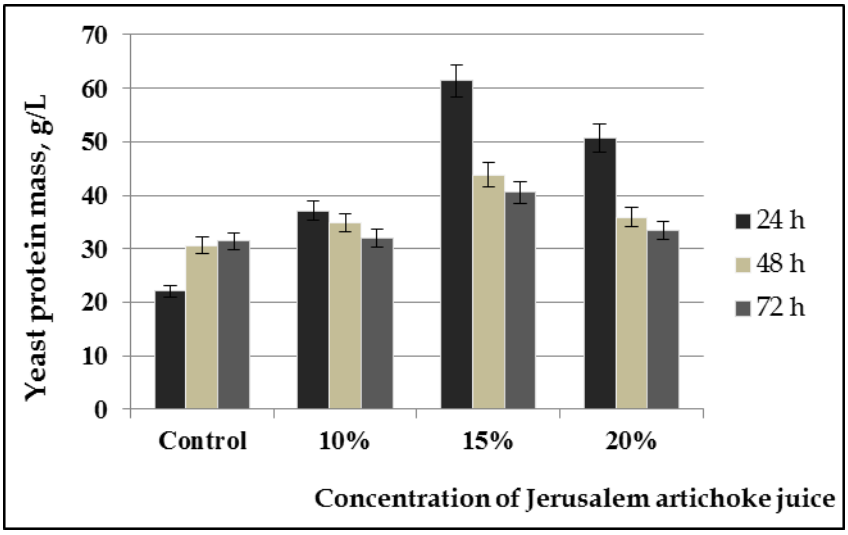

Fig.2. Effect of concentration juice of artichoke and cultivation time *K. marxianus VKM Y*-1148 on yeast protein mass

The maximum stimulating effect was at a content of juice 15% and the duration of cultivation for 24 h (61.4 g / L), which exceeded the control value (22.1 g / L) in 2.8 times, after 48 and 72 h the stimulating effect decreased to 43% and 29% respectively. At the same time Jerusalem artichoke juice enriches whey with biologically active substances (Group B vitamins, biotin, inulin, macro- and microelements) and increases the biological value of the bioconversion product.

CONCLUSIONS

As a result of the conducted studies it has been shown that juice from fresh Jerusalem artichoke tubers can be used to obtain the biomass of strains *of S. cerevisiae G* and *K. marxianus Y*-1148. K. The use of Jerusalem artichoke juice has a stimulating effect on *K. marxianus* and *S. cerevisiae*. It increases the yield of yeast biomass and enriches it with important biologically active substances. It allows replacing the traditional molasses by the Jerusalem artichoke and by reducing the cost of production by increasing the yield of yeast biomass and by excluding the mineral salts.

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Leila GHOLAMI, Negar HASANZADEH, Abdulvahed KHALEDI DARVISHAN [1]

EFFECT OF SAWDUST ON SPLASH EROSION IN LABORATORY CONDITION

SUMMARY

Splash erosion is caused by the impact of raindrops on the soil surface and leads to the detachment of the soil particles. The most effective measures for reducing soil splash are the use of conditioners to improve soil aggregates stability and/or to deploy physical barriers to minimize raindrop impacts. The present study has been conducted to examine the efficiency of sawdust to reduce splash erosion rate in an 15% gradient slope with sandy-clay-loam soil in the laboratory condition, To achieve the study purposes, the soil were covered by sawdust with the surfaces percentages of 30, 50, 70 and 90% in splash cups were placed on the soil in three replicates for each control and treated conditions. A portable rainfall simulator was used to simulate rainfall with the intensity and duration of 40 mm h^{-1} and 15 min, respectively. The results of splash showed that the sawdust reduced both total and net splash.

Keywords: soil amendments, soil conservation, soil erosion, rainfall simulator.

INTRODUCTION

The splash erosion, as in other forms of soil erosion, is a function of raindrop impact energy and of aggregate stability. The most effective soil splash reduction measures are the use of amendments for improvement and reinforcement of soil aggregates and the deployment of physical barriers to minimize raindrop impacts (Kukal & Sarkar 2010 & 2011, Gholami *et al*., 2012). The sawdust is an organic amendment, that it can splash erosion control and increasing infiltration. Existing wood waste and sawdust in the North of Iran comes from wood residues from primary timber processing mills and wood residues in forest floor (Buchanan *et al*., 2002; Copeland *et al*., 2009). Despite the remarkable effort that has been made in recycling wood residues, a great amount of these materials are still being unused. Sawdust is a carbonaceous organic substance which has a very high carbon to nitrogen ratio (typically C:N in sawdust is 300:1) (Tran, 2005). In a study by Johnson (1944), tomato yield

[1]Leila Gholami (corresponding author: l.gholami@sanru.ac.ir), Negar Hasanzadeh, Department of Watershed Management Engineering, Faculty of Natural Resources, Sari Agricultural Sciences and Natural Resources University, Sari, IRAN; Abdulvahed Khaledi Darvishan, Department of Watershed Management Engineering, Faculty of Natural Resources, Tarbiat Modares University, Noor, IRAN

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was greater in plots where sawdust was applied as a surface mulch than in sawdust incorporated plots in the first year. Lunt (1955) obtained the bulk density decreased, moisture increased and total nitrogen and organic carbon increased in all treatments amended with wood chips as compared to check plots. Allison (1965) indicated the most wood products do not contain a concentration of toxic compounds high enough to appreciably affect their use in agriculture. Bulmer (2000) indicated the sawdust application such as trees with more rate and soil with higher organic matter level and more moisture retention relative to untreated soil.

Sawdust mulch could increase the soil oxygen diffusion rate, maintain a more uniform soil temperature, reduce the surface crusting and soil bulk density, and increase the aeration porosity and soil moisture (Johnson, 1944; Lareau, 1989; Khan *et al*., 2000; Tran, 2005). Robichoud *et al*. (2000) applied the wood chips on changing soil erosion in forest areas. They showed that the soil loss reduced with wood chips application. Elliot and Robichaud (2001) showed that the wood chips with size of large wood chips, small wood chips and mixture of wood chip sizes with rates of 80 % could control soil erosion. Buchanan *et al*. (2002), in plot scale and slope of 55 %, stated that the erosion was not significantly different in the small wood chip treatment toward control plots. Whiles, the soil erosion rates in the large wood chip and mixture of chip sizes had significantly different toward the control treatment. Lory *et al*. (2002) survived the effect of sawdust to pen surfaces on nitrogen losses and stated that the sawdust treatment retained more nitrogen in manure when removed from the pen surface and after composting. Also increasing carbon by sawdust additions reduced nitrogen losses by 21 %.

Applying wood chips and agricultural straw by Foltz and Dooley (2003) showed that the soil erosion significantly reduced in plot scale with 4 m^2 area and slope of 5 %. They stated that the straw and two types of wood chips (width of 4 and 16 mm) and two types had lengths of 60, 120, and 240 mm were equally effective in reducing erosion by over 98 %. Yanosek *et al.* (2006) evaluated performance of wood chips in erosion control with two slopes, two soil textures, and three cover amounts. The soil loss to 70 % was reduced in comparison to bare soil, for all cover amounts tested and among each soil type, slope and flow event. Foltz and Wagenbrenner (2010) evaluated of three wood chips blends at 50 and 70 % ground cover for post-fire erosion control on small plots. There was no difference between application rates of 50 and 70% for either of the rainfall plus concentrated flows tested. León *et al.* (2015) in the Zuera Mountains, near the city of Zaragoza (Spain) evaluated the effect wood chips on splash erosion after wildfire and they stated that the wood chips could decrease splash erosion.

The existing literature studied the effectiveness of sawdust only in soil quality. But studies on the efficacy of sawdust on changing splash erosion in rangeland condition did not record. Therefore, this study aimed to determine the efficiency of sawdust with cover 30, 50, 70 and 90 % on splash erosion in laboratory condition.

MATERIAL AND METHODS

The experiments were conducted using splash cups in Laboratory condition using rainfall simulator. A sandy-clay soil was collected of rangeland, Alborz Mountains, Northern Iran. The collected soil was then prepared to be applied to laboratory condition (Thompson, Beckmann 1959; Loch, Donnollan, 1988; Kukal, Sarkar, 2011). The pH, EC and organic matter of experimented soil were 7.32, 74.1 µmohs cm^{-1} and 1.92 %, respectively.

To achieve the study purpose, the sawdust was selected and tested in the laboratory experiments with the cover of 30, 50, 70 and 90 % and then spreading at the surface of soil (Kukal, Sarkar, 2011, Sadeghi *et al*., 2015a, 2015b).

In this study, three splash cups (Morgan, 1978) were used to measure splash erosion. The splashed sediment samples have been collected from upward and downward segments (Gholami *et al*., 2013, 2014, Sadeghi *et al*., 2015a) of cups in each treated and control plot, with the intensity of 50 mm h^{-1}. The splash sediments were then measured using decantation procedure and oven drying at 105 ^{o}C for 24 h and weighed by means of high-precision scales (0.001 g) (Sadeghi *et al*., 2015b; Gholami *et al.*, 2016).

RESULTS AND DISCUSSION

The results of splash erosion before and after sawdust application with rates of 30, 50, 70 and 90 % are shown in Table 1.

Table 1. Net and Total splash erosion (g m^{-2}) resulted from treated control conditions

Plots	Cup No.	Net Splash	Net Splash
Control	1	102.30	158.68
	2	118.90	195.92
	3	95.79	172.43
Average		105.66	175.68
Standard Deviation		11.92	18.83
30	1	93.08	145.49
	2	85.41	152.93
	3	72.92	109.28
Average		83.80	135.90
Standard Deviation		10.18	23.35
Conservation		20.69	22.64
50	1	91.26	136.04
	2	58.84	89.37
	3	69.22	124.96
Average		73.11	116.79
Standard Deviation		16.56	24.38
Conservation		30.81	33.52

70	1	64.04	111.64
	2	59.21	88.32
	3	83.36	131.92
Average		68.87	110.63
Standard Deviation		12.78	21.82
Conservation		34.82	37.03
90	1	78.38	79.69
	2	61.42	98.86
	3	49.59	112.32
Average		63.13	96.96
Standard Deviation		14.47	16.40
Conservation		40.25	44.81

The results of Table 1 show that the sawdust could decrease splash erosion (Gholami *et al*., 2013; Sadeghi *et al*., 2015a, 2015b) the decreased rates were from 21.57 to 46.65 % in net splash erosion from 22.64 to 44.81 % in total splash erosion. The sawdust had the significant effect on reduction of net and total splash erosion at level of 95 and 99 percent, respectively. The reduction of splash erosion using sawdust could finally decrease soil erosion, Robichaud *et al*., (2000), Elliot, Robichaud (2001), Foltz, Wagenbrenner (2010) and León *et al*. (2015) stated the wood chips could control soil erosion. The results of statistical comparison by applying paired t-Test also verified the significant difference between treated and control plots (p=0.001). The results also showed that the sawdust had significant effect in short period after applying on splash erosion. Yanosek *et al*. (2006) and Gholami *et al*. (2013) also reported that the rice straw and wood chips were impressive in a short period.

CONCLUSIONS

The present study was conducted for the study of sawdust effect on splash erosion in laboratory condition. The results showed that the sawdust could protect the soil aggregates from direct impact energy of raindrops. When splash erosion is reduced, the amount of erosion declined and consequently sediment yield decreased. It can finally be mentioned that the sawdust can provide good effects in reduction of splash erosion.

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Dragana LALEVIĆ,
Miodrag JELIĆ, Milan BIBERDŽIĆ [1]

EFFECT OF GENOTYPE AND YEAR ON THE PARAMETERS OF WINTER TRITICALE PRODUCTIVITY CULTIVATED IN NORTHERN MONTENEGRO

SUMMARY

This paper presents the results of the impact of fertilization and genotype on fertility parameters of winter triticale. The experiment tested five genotypes of winter triticale (Odisey, Kg-20, Triumph, Rtanj and Tango) from different breeding. The genotypes were grown in the north of Montenegro, in Bijelo Polje (Sutivan) over three growing seasons (2009-2012). The researches were carried out on the river alluvium soil type on an experiment designed by randomized block design with three replications.

The assessment of winter triticale genotypes was based on the analyzed quantitative and qualitative indicators of productivity (number of grains per spike, 1000 grain mass, hectoliter mass and grain yield). In all three years of the study, genotype Tango had the highest grain yield (5.99 t ha^{-1}), while the lowest yield was observed in genotype Kg-20 (4.48 t ha^{-1}). Also, Tango genotype had the highest value of 1000 grain mass (50.9 g), while the highest hectoliter mass was achieved in genotype Triumph (71.66 kg). The results showed that meteorological conditions, in the three tested years, had a great influence on the yield and quality. The lack of precipitation and extreme temperatures in the third year caused the abrupt termination of grain filling stages which led to a decline in yield and grain quality. The results of these studies would be valuable in terms of its growing as forage crop as well as in terms of its breeding for grain quality and productivity.

Keywords: triticale, genotype, fertilization, 1000 grain mass, hectolitre mass, yield.

INTRODUCTION

Although triticale species had a short development being created by a man,it recently became an important cereal in the world occupying about 4 million ha, of which over 70% in Europe. New varieties of triticale are equal or superior to other cultures for grain yield, forage and biomass production for human food, animal feed or industrial applications (Nefir and Tabără, 2011). Modern triticale cultivars show higher yields and good adaptation to different soil and environments than wheat. Also, it is suitable for cultivation at higher

[1]Dragana Lalević, (corresponding author: dragana.lalevic@gmail.com), Miodrag Jelić, Milan Biberdžić, University in Pristina, Faculty of Agriculture, Kopaonička bb, 38219 Lešak, SERBIA
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altitudes, the soils with poorer physical and chemical properties, saline and acidic soils with a pronounced resistance to biotic and abiotic stresses (Villegas et al., 2010).

Technological quality of triticale is a very complex character, which depends on the genetic potential of cultivars, production practices and agro-ecological conditions (Zečević et al., 2010). Djekic et al., (2013) report that the yield and the yield components of winter triticale significantly vary depending on the system of processing, the applied doses of nitrogen, the genotype and the year conditions, as well as their complex characteristics. Many authors have reported positive effect of N fertilization on grain yield (Gibson et al., 2007; Lestingi et al., 2010). Although Kastori et al., (2005) reported that among the elements of mineral nutrition nitrogen plays a major role in increasing the yield, the results expressed by Zečević et al., (2010) and Lalević (2015) show that nitrogen exerts the greatest effect when used together with phosphorus and potassium. The impact of N fertilizers on cereal yield, yield components and grain quality depends not only on the fertilization strategy but also on the weather conditions (Janušauskaitė, Šidlauskas, 2004; Gibson et al., 2007; Pecio, 2010.)

Studying the parameters of fertility of the five selected genotypes of triticale was carried out in order to identify those genotypes achieving the best results in the given production area. Also, the study results on the varieties selected should make a practical and theoretical contribution to the triticale growing technology.

MATERIAL AND METHODS

The three-year investigations were conducted in Sutivan (the Municipality of Bijelo Polje, north Montenegro), on the alluvial type of soil. Three factorial field microtrials were set up in a randomized block system with three replications. The research included five triticale genotypes created in different selection center (Odisey, Kg-20, Triumph, Rtanj and Tango). Common technology for the production of triticale was used in the experiment. Sowing was carried out by manual method in optimal sowing date (October). The other technology of production which was applied in the experiment was standard, with using NPK fertilizer, in combination 120:80:80. Complex NPK fertilizer (15:15:15) was applied and Calcium-ammonium-nitrate (KAN – 27%) was applied as nitrogen fertilizer in early spring.

The total amount of phosphorus and potassium fertilizers, together with one third of nitrogen, were used in the pre-sowing soil preparation, while the remaining amount of nitrogen was applied in one topdressing in the early spring. The harvest was also conducted manually at the stage of full maturity, where the 1000 grain mass was measured, as well as the hectoliter weight, and the grain yield was corrected for 14% moisture.

The average results of the yield of dry grain, hectoliter weight, 1000 grain mass and protein content, are presented in this paper, for the analyzed period of three years. The obtained results were statistically processed using method of

variance analysis, whereby the significance of average treatments was tested with LSD test, with significance threshold of 1 and 5%.

RESULTS AND DISCUSSION

Soil and climatic conditions

The soil on which the experiment was performed is weakly calcareous, the total content of carbonate being 2.4 – 2.44 %. The humus content in the surface layer of the soil is high (3,35-3,96%), while substitutional and total hydrolytic acidity are quite big (pH u H_2O=6,61-5,53 u KCl=5,01-4,94). The soil is poor with available phosphorus (5.12 – 4.24 mg/100g soil) and potassium (7.5 – 3.8 mg/100g soil). The data in Table 1. for the research period (2009-2012) show that the years, in which the research was carried out, differ in meteorological conditions from the long-term average characteristics of Bijelo Polje.

Table 1. Meteorological data for Bijelo Polje

Year	Months										Sum
	X	XI	XII	I	II	III	IV	V	VI	VII	
Monthly rainfall (mm)											
2009-10	135	94	94	101	80	70	78	80	63	86	**881**
2010-11	65	131	147	36	76	31	46	121	33	79	**765**
2011-12	36	7	55	79	183	57	47	46	34	8	**552**
1961-90	80	115	91	87	68	60	70	76	72	64	**783**
Average monthly temperatures (°C)											
2009-10	9.77	5.95	4.06	1.31	2.4	6.39	10.93	15	18.11	20.95	**9.5**
2010-11	10.12	8.54	2.05	-0.65	0.94	6.03	10.54	14.5	18.9	21.23	**9.2**
2011-12	9.3	3.25	2.17	-1.72	-3.52	5.96	10.8	15.02	20.67	24.63	**8.7**
1961-90	9.4	4.7	0.2	-1.3	0.7	4.9	9.0	13.3	16.3	17.9	**7.5**

The average air temperature in all three years of the study was higher compared to the multiannual average, while the rainfall in 2009/10 was higher for 98mm, and lower in 2010/11 and 2011/12 for 18mm, or 213mm in relation to the multiannual average. In the first year of study (2009/10), the average air temperature was increased by 2.0^0C compared to the average of several years. The average air temperature in 2010/11 and 2011/12 was 1.7^0C and 1.2^0C higher in comparison to the perennial average. Considerable less rainfall in November 2011, heavy snowfall in February 2012 and significantly higher air temperature in July of the same year, adversely affected the development of plants which resulted in lower yields compared to the first two years of the study.

The data about the achieved number of grains per spike, 1000 grain mass, grain density and grain yield of the studied genotypes of triticale, during the three-year test period, are shown in Table 2. The results of our study show that the genotype and meteorological conditions during the research, expressed different character and intensity of the observed effects on productivity parameters. Numbers of grains per spike represent quite a trait that is largely dependent on agro-ecological conditions, and the applied agro-technology (Jaćimovic et al.,2012). The average values show that the lowest number of

grains per spike had genotype Triumph (28.3), while the largest number of grains per spike had Odysseus (36.3). With the exception of genotype Tango, where no statistically significant difference was noted, genotype Odysseus compared to others involved in the study had a larger number of grains per spike and the difference was at 0.01 level of significance. The analysis of the average number of grains per spike of the tested genotypes of triticale in the observed years, regardless of the genotype, shows that the lowest average number of grains per spike was in 2010/11 (31.4), and the highest in 2011/12 (33.7)

Table 2. Values of the tested productivity parameters of winter triticale

		Year		
Variety	**2009/10**	**2010/11**	**2011/12**	**Average**
Number of grains per spike				
Odisej	31,8	32,1	45,5	36,5
Kg-20	30,8	31,8	30,0	30,9
Trijumf	28,1	27,2	29,7	28,3
Rtanj	34,0	34,7	28,6	32,4
Tango	41,8	31,8	34,8	36,2
Average	**33,3**	**31,4**	**33,7**	**32,9**
LSD 0,05	1,277	1,623	1,551	2,900
LSD 0,01	1,811	2,303	2,199	4,113
1000 grain mass (g)				
Odisej	49,3	42,5	46,3	46,1
Kg-20	38,5	35,0	33,6	35,7
Trijumf	48,3	42,7	45,0	45,3
Rtanj	49,7	47,3	44,3	47,2
Tango	54,1	49,6	48,8	50,9
Average	**47,9**	**43,4**	**43,6**	**45,0**
LSD 0,05	0,859	1,271	0,975	1,436
LSD 0,01	1,218	1,802	1,383	2,036
Hectoliter weight (kg)				
Odisej	73,72	69,35	69,55	70,87
Kg-20	66,79	63,65	64,85	65,09
Trijumf	75,64	70,55	68,80	71,66
Rtanj	65,86	64,39	67,10	65,78
Tango	71,09	69,30	66,30	68,90
Average	**70,62**	**67,45**	**67,32**	**68,46**
LSD 0,05	0,985	0,819	1,312	2,175
LSD 0,01	1,394	1,161	1,605	3,070
Grain yield (t ha^{-1})				
Odisej	5,51	4,97	4,08	4,85
Kg-20	5,25	4,45	3,79	4,48
Trijumf	6,16	5,37	4,56	5,37
Rtanj	5,96	5,62	4,49	5,36
Tango	6,95	6,05	4,97	5,99
Average	**5,96**	**5,29**	**4,38**	**5,21**
LSD 0,05	0,117	0,107	0,093	0,342
LSD 0,01	0,166	0,152	0,132	0,485

During the three-year research, genotype Tango had the highest average 1000 grains mass (50.9g). The average three-year value of a 1000 grain mass in genotype Tango was very significantly higher compared to the values observed in other tested genotypes. The highest 1000 grain mass in tested genotypes of triticale was achieved in the first year of study (47.9g), while the lowest was in the second year of study (43.4g). The average values of the 1000 grains mass obtained in this study were somewhat higher compared to the values of this characteristic obtained by Kondić (2009), Lalević et al., (2012); Biberdžić et al., (2013). According to Milošev (2000) the 1000 grains mass is the result of complex interactions of varietal specificity, agroecological conditions and the applied technology.

After reviewing the average values of hectoliter weight of the tested genotypes of triticale, regardless of the years, it can be concluded that genotype Kg-20 (65.09 kg) had the lowest average hectoliter weight, while the highest value of the tested characteristics was achieved in genotype Triumph (71.66 kg). The analysis of the average value of hectoliter weight of the tested genotypes of triticale in the observed years, regardless of the genotype, show that the lowest average hectoliter weight was in the third year of study (67.32 kg), while the highest was in the first year of study (70.62 kg). The impact of the year on the value of hectoliter weight was also noticed in our research. Accordingly, in the first year, which was the most favorable according to climatic conditions, the highest average value of the hectoliter weight was recorded (70.62 kg). Numerous authors (Jelić et al., 1998; Stošović et al., 2010; Lalević et al., 2012) have confirmed in their earlier researches that weather conditions may affect the value of hectoliter weight.

The grain yield per unit area is one of the most important factors affecting the profitability and efficiency of production. Several factors are decisive in triticale yields increasing: the genotype, cultural practices, agro ecological conditions, local climatic and soil characteristics, mineral nutrition and adequate protection from plant diseases, pests and weeds (Milovanović et al., 2011).

Genotype Kg-20 had the lowest average grain yield (3.79 t ha^{-1}) in 2011/12, while Tango had the highest average grain yield (6.95 t ha^{-1}) in 2009/10.

The analysis of the average grain yield of the tested genotypes of triticale in the observed three-year period was highest in genotype Tango (5.99 t ha^{-1}). At the same time it was significantly much higher in comparison to other tested genotypes. The analysis of the average grain yield of the tested genotypes of triticale in the observed years, regardless of the genotype, show that the highest average yield of the tested genotypes of triticale was in the first year (5.7 t ha^{-1}), while the lowest yield was in the third year of study (4.5 t ha^{-1}). Taking into account the agro-climatic characteristics of the area, which cause the later sowing and later maturing, as well as the average yields that triticale achieves in commercial production, these results are satisfactory for this area. Considerable variation in grain yield depending on the impact of the genotype and the year of

research was noted by Stošović, 2009; Biberdžić et al., 2012, 2013; Lalević et al., 2012; in their researches.

CONCLUSIONS

The results of the research of five genotypes of winter triticale in the period 2009-2012 in the north of Montenegro, show that:

- Agroecological conditions of the year and genotype had significant effect on yield and quality characteristics of triticale.
- The lowest number of grains per spike had genotype Triumph (28.3), while the highest number of grains per spike had Odysseus (36.3).
- The highest average value of a 1000 grain mass during the three years of research had variety Tango (50.9g) .
- Variety Triumph in the first and second year of the study achieved the highest value of hectoliter weight, while in the third year the highest value of hectoliter weight had variety Odisey.
- The average yield of tested genotypes of triticale was highest in the first and lowest in the third year of the study.
- In all three years of study the highest grain yield had genotype Tango.

Based on these results, we can conclude that the genotypes Tango, Triumph and Rtanj showed a good adaptability in both parameters of productivity and as such can be recommended in a commercial triticale production in agroecological conditions of northern Montenegro.

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Oksana YEREMENKO,
Svitlana KALENSKY, Valentina KALYTKA [1]

SAFFLOWER PRODUCTIVITY DEPENDING ON SEED TREATMENT BY AKM PLANT GROWTH REGULATOR AND LEVEL OF MINERAL NUTRITION

SUMMARY

Extremely uneven distribution of moisture during the growing season dramatically increases not only the risk of safflower seeds yield reduction, but its quality as well. One of the main reserves of solving this problem is the dose of fertilizers, particularly nitrogen, and use of plant growth regulators (PGRs) in the critical phases of plant development. It is the optimum combination of these factors that is a significant reserve for increasing yield stability and quality of safflower seeds. The aim of the research was to identify the impact of AKM PGR on productivity of safflower plants at different levels of mineral fertilization. The results of the study on the impact of the AKM plant growth regulator on growth, development and yield formation of safflower in low moisture conditions of Southern Steppe of Ukraine are presented. Optimal concentration of AKM PGR (0.0015 g/l) was determined. Seeds, processed by AKM PGR, had laboratory germination 5.7 % higher than in the control. In 2016, the safflower plant height for all variants was higher than this figure in the other years of the study. This is because HTC in 2016 for the period BBCH 00-39 was higher than in 2015 by 1.4 times. Therefore 1 anthodium formed on average 13.2 to 21.6 seeds. AKM PGR increased, compared to control, both weight of seeds in 1 anthodium, and the number of anthodia during the study years, thanks to anti-stress properties of AKM. This preparation significantly affected yield only in unfavourable years, and the impact of fertilizers was insignificant. The AKM plant growth regulator is recommended for the use on safflower in 0.0015 g/l concentration.

Keywords: Safflower, Productivity, Plant growth regulator, Growth and development of the plants, Stress resistance.

INTRODUCTION

In recent years, the global food market has increased demand for vegetable fats (Ermakov and Polyakov, 2013). Among oilseed crops, sunflower and safflower are of particular importance in food industry (Adamen *et al*., 2014).

[1]Oksana Yeremenko, (corresponding author: ok.eremenko@gmail.com), Svitlana Kalensky, National University of Life and Environmental Sciences of Ukraine, UKRAINE, Valentina Kalytka, Tavria State Agrotechnological University of Ukraine, UKRAINE
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Their growing is closely connected with the increase in oil production that must be reached through higher yield. This, however, entails a number of problems (Ermakov and Polyakov, 2014, Dordas and Sioulas, 2009).

Crop production depends on weather conditions throughout the entire growing season. Weather risks, significantly affecting crop yields, are one of the least predictable and objective factors (Quiroga, 2001, Bezditko, 2011). Therefore, safflower seed production in many farms of the Steppe zone of Ukraine is characterized by reducing yield and its stability and, consequently, raising production costs (Dudnik and Chomyak, 2008).

There are two ways to enhance plant resistance to stresses: by selecting resistant varieties or hybrids at the genetic level or managing resistance via technological methods. Increasing plant resistance to stresses under differentiated use of plant growth regulators (PGRs) at the various growth stages is an effective way to increase yield (Svetlova *et al*., 2002).

Raising crop productivity under the PGR influence is associated with the increase in plant adaptation to the growing conditions and intensification of cell activity in the plant organism due to stimulation of biochemical processes thereby leading to the optimization of nutrition, respiration and photosynthesis processes (Klimenko, 2015). PGRs facilitate the higher realization of genetic potential of plants (Kalytka and Zolotuhina, 2011).

Mohsen Janmohammadi claims that silicon nanoconcentrations (nSiO2) increase leaf surface area by 48% and stimulate growth processes of safflower under adverse weather conditions and against various organic and mineral nutrition (Janmohammadi, 2016).

According to a group of scientists including Sibgha Noreen, Muhammad Ashraf, Mumtaz Hussain, Amer Jamil, Kazem Ghassemi-Golezani, and Ayda Hosseinzadeh-Mahootchi the use of salicylic acid to grow sunflower and safflower reduces the negative impact of stress factors by increasing activity of antioxidant enzymes. In addition, the processes of growth and photosynthetic activity of plants are also activated (Noreen *et al*., 2009, Kazem Ghassemi-Golezani and Ayda Hosseinzadeh-Mahootchi, 2015).

Fertilizers provide different effects on growth and development of safflower (Omid Mohsennia and Jalal Jalilian, 2012; Mostafa Heidari and Sepideh Mohamadi, 2014). The authors have established that the nitrogen in an amount of 150 kg/ha increased yield by 55% compared to control. With increasing this dose to 225 kg/ha the yield increased by only 23%. Different fertilizer norms influence not only the yield but also the seed quality, in particular fatty acid composition of seeds.

The efficiency of PGRs and mineral fertilization of oilseed crops under conditions of sufficient moisture and observation of cultivation technology is rather high (Bonfim-Silva *et al*., 2015). At the same time, few studies elucidate the cultivation of safflower with the use of PGRs and different mineral fertilization in the conditions of insufficient and unstable moisture. Thus, poor knowledge of this issue has led to the direction of our research.

The development of agricultural techniques for growing safflower has become a pressing issue in arid conditions of South Steppe of Ukraine. The aim of this research is to develop and justify the elements of safflower cultivation technology capable to ensure for agrocenoses the level of productivity close to the genetic potential, given the growth and development patterns of plants.

MATERIAL AND METHODS

Field studies were carried out over the period 2014-2016 in the scientific-production centre of Tavria State Agrotechnological University (Melitopol district, Zaporizhzhia region, Ukraine), and laboratory studies – in the Laboratory for the Soil and Crop Production Quality Monitoring (Scientific Research Institute of Agrotechnology and Ecology at TSAU).

The soil of study plots was represented by chernozem containing 2.91% of humus, 81.5 mg/kg of light hydrolyzed nitrogen, 138.4 mg/kg of movable phosphorus, and 165.8 mg/kg of exchangeable potassium as a weighted average; pH of the soil solution was close to neutral.

Soil moisture conditions varied between years both in the amount of rainfall and evenness of its distribution. The least rainfall in the vegetation period was recorded in 2015 (155 mm; HTC = 0.56), and the highest one - in 2014 (233 mm; HTC = 0.81). The year of 2016 had uneven distribution of rainfall (HTC = 0.67) and high temperatures; minimum relative air humidity in the flowering season constituted 35.5%. In general, agrometeorological conditions in 2015, compared to 2014 and 2016, were more optimal in terms of minimum relative air humidity in the flowering season (45.8 %) and evenness of rainfall distribution.

The agricultural techniques in the experiment were common for the Steppe zone of Ukraine except for the studied factors. The total size of an elemental area was 90 m^2, count area – 54 m^2. The effects of mineral nutrition (factor A), presowing AKM treatment of seeds (factor B) and agrometeorological conditions of the year (factor C) on the formation of safflower yield structure were studied in a three-factor field experiment according to the scheme (Table 1).

Table 1. Field research scheme (2014-2016)

Fertilizer system, kg/cwt a.s. (factor A)	PGR (factor B)	Agrometeorological conditions of the year (factor C)
Control (without fertilizers)	without PGR	2014 – 2016
	AKM	
$N_{45}P_{60}K_{45}$	without PGR	
	AKM	

The AKM formulation with an application rate of 0.33 l/t was used. The seeds were incrusted 1-2 days before sowing (10 litres of working solution per 1 ton of seeds).

AKM is a semi-synthetic film-forming plant growth regulator with the anti-stress effect allowed for the treatment of seed and spraying of vegetating plants of cereals, legumes, oil-bearing and vegetable crops, and hops. The

formulation includes dimethyl sulfoxide (0.0018 - 1.8 g/l), butylhydroxytoluol (0.0027 - 2.7 g/l), PEG 1500 (440 g/l), PEG 400 (190 g/l), and the rest is water (Kalytka V.V. *et al*., 2011).

The seed quality was assessed by the germination energy and laboratory germination capacity of the seeds sown in paper rolls according to the standard method. Crop tending, census and monitoring of the plant growth and development, and observations of the formation of structural elements of safflower yield were performed according to Rozhkov (Rozhkov, A.O. *et al*., 2016). The results were mathematically processed using Student's t-test with the assistance of the Agrostat software program.

RESULTS AND DISCUSSION

Presowing seed treatment of safflower activates self-regulation processes and provokes the increase in germination and resistance to adverse environmental factors. It should be noted that the AKM effect depends on the concentration of active substances (butylhydroxytoluol, dimethyl sulfoxide).We found out that, depending on different concentrations of active substances, the AKM incrustation of safflower seeds leads either to stimulation or inhibition of germination (Fig.1). Maximum laboratory germination of safflower seeds was recorded with using an AKM dose of 0.0015 g/l (by active substance) that exceeded control by 5.7%.

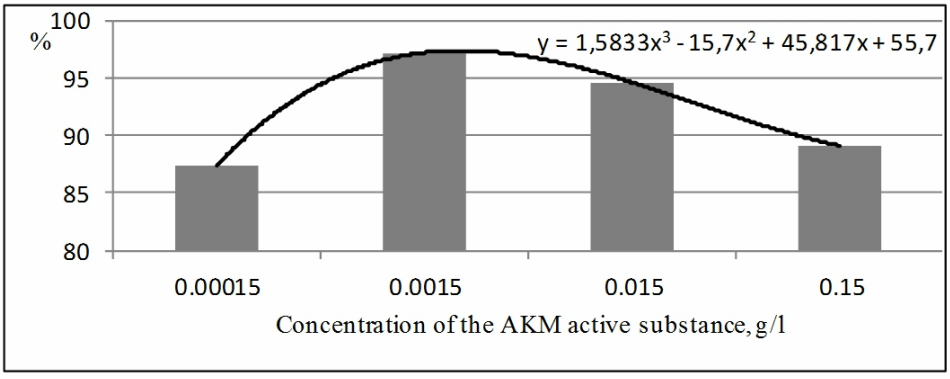

Figure 1. Dependence of laboratory germination of safflower seeds on concentrations of the AKM active substance: - Approximating polynomial curve of the 4th order

Another essential advantage of AKM in a.s. concentration of 0.0015 g/l is insignificant difference between germination energy and germination of seeds which in situ reduced the period between sowing and emergence of shoots by 1-2 days and helped to obtain more evenly distributed shoots. It is the reason why in the field experiment we treated seed with this concentration of AKM.

Sowing conditions for safflower were favourable during the studied years. In the sowing-shoots period (BBCH 00-09), HTC ranged from 1.43 (2016) to

1.71 (2014), and the field germination did not vary significantly between the studied variants (Table 2).

Table 2. Field germination and biometrical indices of growth and development of safflower*

Norm of fertilizers, kg/ha a.s. (factor A)	PGR (factor B)	Agrometeorological conditions of the year (factor C)	Index		
			Field germination, %	Plant height, cm	Leaf surface area, thou. m^2/ha
K (no fertilizers)	without PGR	2014	83.6	62.3	18.35
	with PGR		88.4	69.5	26.14
$N_{45}P_{60}K_{45}$	without PGR		85.7	68.5	25.61
	with PGR		89.3	73.4	27.59
K (no fertilizers)	without PGR	2015	85.2	63.1	19.52
	with PGR		89.7	71.4	27.08
$N_{45}P_{60}K_{45}$	without PGR		87.1	69.4	24.25
	with PGR		90.2	70.8	26.94
K (no fertilizers)	without PGR	2016	85.9	82.6	21.35
	with PGR		90.2	90.1	29.64
$N_{45}P_{60}K_{45}$	without PGR		89.3	88.5	30.28
	with PGR		89.9	89.6	32.59
LSD_{05} of partial differences, for the factor: A				0.32	0.71
B				0.47	0.68
C				0.38	1.02

*- biometrical indices are given for the flowering stage of safflower (BBCH-65)

The AKM effect accelerated the growth and development of safflower plants, and all the growth stages in the variants with PGR started 3-4 days earlier as compared to control.

In 2016, the height of safflower plants in all the variants exceeded the height values recorded in other studied years. This is due to the fact that HTC in 2016 for the period BBCH 00-39 was 1.4 higher than HTC in 2015.

Leaf surface area of safflower crops had the largest range of fluctuations in the flowering stage, reaching its peak (32.59 thousand m^2/ha) in 2016 in the variant with the AKM presowing seed treatment and mineral fertilization.

The indices of safflower productivity elements varied depending on the studied agricultural techniques. This, the least number of anthodia per one plant was recorded in control for the entire research period (Table 3). It was 1.3-1.5 times greater in the variant with the AKM presowing seed treatment and mineral fertilization. However, in case of the PGR presowing seed treatment or mineral fertilization, taken alone, this index increases by 1.2-1.4 times.

The number of seeds in the anthodium, weight of 1000 seeds and the weight of seeds in the anthodium in the variant with the AKM presowing seed treatment and mineral fertilization 1.1-1.5 times exceeded control values. Depending on the studied factor, one anthodium on average contained from 13.2 to 21.6 seeds.

Changes in the growing conditions of safflower influenced its yield depending on the used agricultural techniques. For three years of research the highest yield was recorded in 2016 in the variant with the AKM presowing seed treatment and mineral nutrition (2.38 t/ha). The effect size of the studied factors on the yield equalled to A – 21.9 %, B – 32.1 %, C – 27.3 %, and BC – 11.7 %.

Under the studied agricultural techniques the safflower realizes its genetic potential by 83- 95%. Thus, we advise agroproducers to grow safflower according with the developed technology, especially in zones of insufficient and uneven moisture, as a high-intensive plant, alternative to sunflower.

Table 3. Structural elements of safflower yield depending on the studied factors (2014-2016)

Norm of fertilizers, kg/ha a.s. (factor A)	PGR (factor B)	Agromet. conditions of the year (factor C)	Index				
			Number of anthodia per one plant	Number of seeds in the anthodium	Weight of 1000 seeds (g)	Weight of seeds in 1 plant (g)	Yield, t/ha
K (no fertilizers)	without PGR	2014	7.6	13.2	37.4	3.92	1.04
	with PGR		10.7	19.6	39.5	8.28	1.75
$N_{45}P_{60}K_{45}$	without PGR		9.9	19.8	40.8	7.99	1.69
	with PGR		11.4	20.2	41.6	9.58	1.87
K (no fertilizers)	without PGR	2015	8.3	18.9	41.6	6.53	1.44
	with PGR		10.6	21.6	43.2	9.89	2.18
$N_{45}P_{60}K_{45}$	without PGR		10.9	21.5	42.1	9.87	2.16
	with PGR		11.7	21.4	43.6	10.94	2.21
K (no fertilizers)	without PGR	2016	9.6	18.9	36.4	6.60	1.45
	with PGR		11.2	21.2	40.5	9.62	2.21
$N_{45}P_{60}K_{45}$	without PGR		11.5	21.1	40.9	9.92	2.08
	with PGR		12.8	21.3	41.7	11.37	2.38
LSD_{05} of partial differences, for: A			0.4	0.8	0.4	0.3	0.12
B			0.3	0.6	0.6	0.3	0.18
C			0.3	1.0	0.5	0.4	0.19

CONCLUSIONS

Under different concentrations of active substances, the incrustation of safflower seeds with AKM leads either to stimulation or inhibition of germination. Maximum laboratory germination of safflower seeds was recorded using an AKM dose of 0.0015 g/l (active substance) that exceeded control values by 5.7%. In the period between sowing and emergence of shoots (BBCH 00-09), HTC ranged from 1.43 (2016) to 1.71 (2014) and, therefore, the indices of field germination did not vary significantly between the studied variants in 2014-2016.

In 2016, the height of safflower plants in all the variants exceeded the height recorded in other years of research.

Maximum leaf surface area (32.59 thousand m^2/ha) was in 2016 in the variant with the AKM presowing seed treatment and mineral fertilization. In the stage of the formation of generative organs (BBCH 15–51) HTC ranged from 0.84 (2015) to 1.38 (2014). Depending on the studied factor, one anthodium formed on average from 13.2 to 21.6 seeds. The effect size of the factors on safflower yield equalled to A – 21.9 %, B – 32.1 %, C – 27.3 %, a BC – 11.7 %.

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Messaouda BELAID, Farida BENZINA,
Fatma ACHEUK, Khadidja ADJOU, Houria OSMANE BACHA [1]

BACTERIAL CONTAMINATION OF HAEMOLYMPH IN EMERGING WORKER HONEYBEE (*APIS MELLIFERA* L) PARASITIZED BY VARROA DESTRUCTOR

SUMMARY

Varroa destructor is an obligatory ectoparasite of the honeybee (*Apis mellifera* L). The mites use their piercing month parts to suck out hemolymph from immature and adult bees caused direct damage (morphological, physiological abnormalities) and indirect damage due to microbial pathogens. The aim of this work was to research the bacterial microflora in hemolymph of emerging healthy and parasitized worker honeybee by *Varroa destructor*. For the isolation and identification of the bacteria, the morphological and biochemical tests were done. The results showed that the haemolymph of the healthy worker (*Apis mellifera* L) is free of bacteria. When the V.*destructor* breaks the cuticle, the microorganisms invade the heamolymph. The infested worker honeybees harbored a total 9 strains belonging to 6 strains of Bacillacea (66,66%), 1 of Peanibacillacea (11,11%) and 2 of Enterobacteriacea (22,22%). Based on the Gallery API 20 E (Bio-Merieux), the genera of Bacillacea and Peanibacillacea included *Bacillus licheniformis* (4 strain), *Bacillus mycoide* (1strain), *Bacillus coagulans* (1 strain) and *Brevibacillus chohinensis* (1 strain) respectively. *Bacillus licheniformis* was for probably the most frequent species. The Enterobacteriaceae included *Aeromonas hydrophila* and *Pantoa sp.*

Keywords: Honeybee, *Apis mellifera* L, *Varroa destructor*, bacterial contamination. hemolymph.

INTRODUCTION

Varroa destructor (Anderson and Trueman, 2000) an obligatory ectoparasite of the honeybee (*Apis mellifera* L), has caused severe damage to populations of this species world-wide in recent years (Le Conte *et al*., 2010). The direct negative effect of the *Varroa* on honeybee has been well documented (Weinberg and Madel, 1985; Daly *et al.*, 1988, Wienands and Madel, 1988; Marcangeli *et al*., 1992; Bowen-Walker and Gunn, 2001; Contzen *et al.*, 2004; Yang and Cox-Foster, 2005; Belaïd and Doumandji, 2010; Belaïd *et al*., 2017). However, in the recent years, scientists have diverted their attention towards the indirect effect by virus transmission, the foulbrood diseases and fungal infection (Hrabak, 2003;

[1]Messaouda Belaid (corresponding author: belaidfo@yahoo.fr), Farida Benzina, Fatma Acheuk, Khadidja Adjou, Houria Osmane Bacha, University of Boumerdes, Faculty of Science, Department of Biology, M'Hamed Bougara, ALGERIA.
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Benoit *et al*., 2004; Tentcheva *et al*., 2004, Hamdi *et al*., 2011). Tentcheva *et al*., (2004) reported that the infection of bees with the Deformed Wing Virus (DWV) was strongly linked to the presence of *Varroa*. De Rycke *et al*., (2002) reported that *Varroa destructor* was capable of transporting spores of *Paenibacillus larvae* (the American foulbrood agent) to the surface of its body, thus allowing the parasite to participate in its propagation. The Fungi or spores of fungi are found on the surface of *V. destructor* (*Aspergillus flavus*, *Penicillium multicolor*, *Penicillium simplicissimum*, *Mucor ramosissimus*, *Mucor indicu*, *Mucor hiemalis* and *Ascosphaera apis* (Benoit *et al*., 2004). The cuticle itself constitutes an excellent barrier against parasite invasion. However, the damaging host integument during the feeding behavior of *Varroa* rendered the bees vulnerable to the microbial infections (Kanbar and Engels, 2005). To our knowledge, there are no reports about the bacterial microflora in heamolymph of honeybee parasitized by *Varroa destructor*. In this paper, the bacteria enable to be transmitted into honeybee haemocoel was investigated.

MATERIAL AND METHODS

Healthy and parasitized preemerging honeybees (*Apis mellifera* L) were collected from the brood of the apiary of Tizi Ouzou in early summer 2015. 1 µl of haemolymph of the samples were diluted in 9µl sterile normal saline (1:10 µl) immediately vortexed, then the haemolymph solution was plating on nutrient agar plates with the help of sterilized loop and incubated at 37° C. The plates were prepared in duplicate. Each different colony was subcultured to obtain pure culture. Selected strains were initially characterized by cell morphology and Gram's, endospore staining using the standard procedures. Primary identification was carried out according to Bergey's Manual of systematic Bacteriology (Holt *et al*., 1994). Biochemical characteristics were tested with API 20E galleries (Biomerieux).

RESULTS AND DISCUSSION

The preliminary results about the occurrence of bacteria microflora in haemolymph of emerging healthy and parasitized worker honeybee (*Apis mellifera intermissa*) by *Varroa destructor* were shown in table 1, table 2 and Fig 1 (A, B).

The results showed that the haemolymph of the healthy emerging working bee was free of bacteria. As seen in table 1 and Fig 1A, the infested worker honeybees harbored 9 strains belonging to 6 strains (S1, S2, S3, S4, S5 and S6) of Bacillacea (66,66%), 1(S7) Peanibacillacea (11,11%) and 2 (S8 and S9) Enterobacteriacea (22,22%). The bacteria Gram-positive rods endospore forming aerobes or facultative anaerobic isolated from the haemolymph of parasitized samples were classified according to Bergy in the family Bacillacea (S1, S2, S3, S4, S5 and S6). As seen in table 2 and Fig 1 B, 6 strains were isolated from the

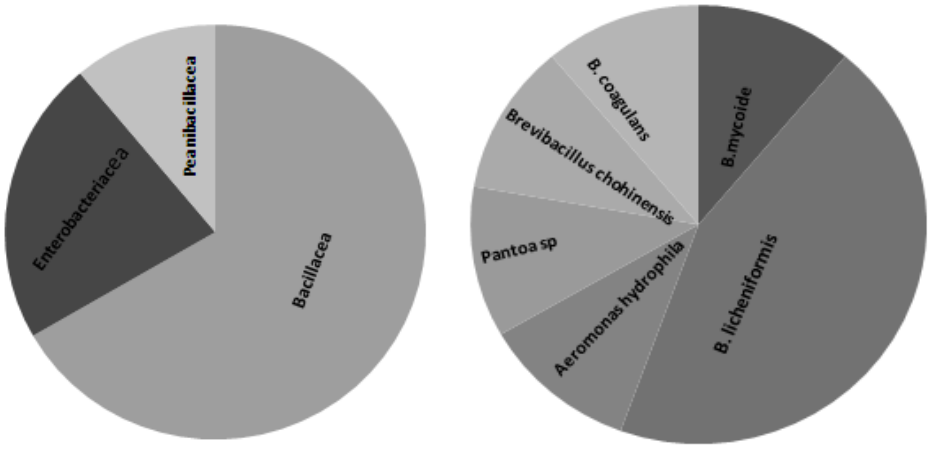
haemolymph. The *Bacillus licheniformis* was for probably the most frequently species (44,44%) with 4 strains (S2, S3, S4 and S5) followed by *Bacillus mycoide* (1strain: S1*)* and *Bacillus coagulans* (1strain: S6) representing 11,11% of each one. In our study, generally, *Bacillus* sp was positive for the Vogues proskaeur reaction (VP). The isolate bacteria could produce catalase and oxidase. Also, the *Bacillus* sp was capable of using glucose, mannitol, arabinose as carbohydrates sources for growth. In our study, *Bacillus* sp was well represented in our data. One strain of *Brevibacillus chohinensis* (S7) was observed. The isolate was identified as Gram-positive, aerobic, spore-forming *Bacillus*.

According to Bergy's, the strains (S8 and S9) Gram-negative oxidase-positive rods non-sporulating facultative anaerobic capable of fermenting glucose were identified as members of the family Enterobacteriacea. Based on the Gallery API 20 E (Bio-Merieux), the genera included *Aeromonas hydrophyla* (11,11%) and *Pantoa sp* (11,11%) for strains (S8 and S9) respectively.

Table1. Biochemical characteristics of isolated bacteria from the haemolymph in emerging worker honeybee parasitized by *V. destructor*.

	S1	S2	S3	S4	S5	S6	S7	S8	S9
Ortho-Nitro-phénol-Galactosidase (ONPG)	-	+	+	+	+	-	-	+	-
Arginine Di-Hydroxylase (ADH)	+	+	+	+	+	-	-	+	-
Lysin Di-Carboxylase (LDC)	+	+	+	+	+	-	-	-	-
Ornithine décarboxylase (ODC)	-	+	+	+	+	-	-	-	-
Citrate utilization test (CIT)	+	+	+	+	+	-	+	-	-
H_2S Production test	-	-	+	-	-	-	-	-	-
Uréase (URE)	+	+	+	-	-	-	-	-	-
Tryptophane Désaminase (TDA)	+	+	+	+	+	-	-	-	-
Indol production (IND)	-	+	-	+	-	-	-	-	-
Acetoin production (VP)	+	+	+	+	+	+	+	+	+
Gelatinase (GEL)	+	+	+	+	+	-	-	+	-
Glucose (GL)	+	+	+	+	+	+	+	+	+
Mannose (MANE)	-	+	-	+	-	-	+	+	+
Inositol (INO)	+	+	-	-	+	-	-	-	-
Sorbitol (SOR)	-	-	-	+	+	-	+	-	+
Rhamnose (RHA)	-	+	-	+	-	+	-	+	+
Saccharose (SAC)	-	+	-	-	-	+	+	-	+
Melibiose (MEL)	+	+	+	+	-	+	+	-	+
Amydaline (AMY)	-	-	+	-	+	-	+	-	+
Arabinose (ARA)	+	+	+	+	+	+	+	+	+
Mannitol (MAN)	+	+	+	+	+	-	-	+	+
Production NO2	+	-	-	+	-	+	-	+	-
N2 Nitrate reduction test	-	+	+	-	+	+	+	-	+
Oxidase test (OXY)	+	+	+	+	-	+	+	+	+
Catalase test (CAT)	-	+	+	+	+	-	+	-	+
Motility (MOT)	-	+	+	+	-	+	+	-	-

(+): positive test ;(-): negative test



A B

Figure 1: Occurrence of bacteria family (A) and species (B) in haemolymph of emerging worker honeybee infested by *V.detructor*

Table 2: The identification probabilities for isolated Strains

		Probability
S1	*Bacillus mycoide*	69,43%
S2	*Bacillus licheniformis*	99,74%
S3	*Bacillus licheniformis*	66,97%
S4	*Bacillus licheniformis*	88,65%
S5	*Bacillus licheniformis*	66,97%
S6	*Bacillus coagulans*	97,95%
S7	*Brevibacillus chohinensis*	79,02%
S8	*Aeromonas hydrophila*	79,31%
S9	*Pantoa sp*	63,25%

In our work, the haemolymph of the healthy emerging worker honeybee was free of bacteria. According to Tubiash *et al.,* (1975), it was generally assumed that the circulatory system of healthy animals was sterile. The body surface of adult honey bees is relatively free of bacteria, likely due to grooming behavior (Gilliam, 1997). According to Keller *et al.,* (2013), the pre-adul honeybee was almost sterile systems. In invertebrates, the circulating haemocyte has a major role in the protection of the animal against aggressive microorganisms by participing in recognition, melanisation, phagocytosis and cytotoxicic activities (Jiravanichpaisal *et al.,* 2006). The presence of bacteria is usually considered to be a sign of disease (Tubiash *et al.,* 1975). *Varroa* bee hive attack is a serious and common problem in beekeeping. The mite gets attached to the body of the bee and brood and weakens the bee by repeated sucking of haemolymph (Vanikova *et al.,* 2015). When the *Varroa destructor* breaks the cuticle, the microorganisms invade the heamolymph (Kanbar and Engels, 2005).

Many scientists increased attention to the microflora of the ectoparasites and their role as vector of viral, fungal and bacterial disease (Ball, 1985; Gliński and Jarosz, 1992; Ball, 1997; Bowen Walker *et al*., 1999; Chen *et al*., 2004; Maddaloni and Pascual, 2015; Vanikova *et al.,* 2015).

To our knowledge, this work was the first report studying the role of the mite as vector to bacterial microflora in the haemolymph of honeybees.

In the study, the results showed that the Bacillacea was the most frequently present in heamolymph of worker honeybees (*Apis mellifera* L) followed by Enterobacteriaceae and Peanibacillacea. According to De Rycke *et al*., (2002), *Varroa destructor* could play a role in the transmission of *Paenibacillus larvae* spores, *Bacillus* (formerly known as *Bacillus larvae*) responsible for American foulbrood from infected to healthy bee colonies. Hrabak (2003) identified the genus *Staphylococcus albus* and *Enterobacter cloacae* associated to the ectoparasite mite. Tsagou *et al*., (2004) isolated bacterial strains from *Varroa destructor* belonging to Bacillacea (*Bacillus sp*) and Micrococcaceae. Hubert *et al*., (2015) found *Morganella* sp, *Enterococcus* sp, *Pseudomonas* sp, *Rahnella* sp, *Erwinia* sp and *Arsenophonus* sp. Maddaloni and Pascual (2015) reported the occurrence of *Bacillus subtilis*, *Pseudomonas syringae*, *Pantoa agglomerans*, *Pantoa vagans*, *Paenibacillus* wynnii, *Staphylococcus caprae, Bifidobacterium asteroids, Staphylococcus caprae* and *Micrococcus luteus* associatetd to *Varroa destructor*. Vanikova *et al*., 2015, recorded *Microbacteruim* sp and *Bacillus* sp. Because microorganisms are ubiquitous in nature, it is not surprising to find a variety of them associated with insects. Generally, the kinds of microorganisms involved with an insect reflect the microflora of the surrounding environment (Ingraham *et al*., 1975). The pathogenicity is largely associated with entry to the hemocoel either through a wound in the exoskeleton or more generally through the peritrophic membrane of the gut (Priest, 2000). Bacteria that fall within the category of insect pathogens, families characteristic of enthomopathogens, are: Bacillaceae, Enterobacteriaceae, Streptococaceae (Hrabak, 2003), Pseudomonadaceae, Lactobacillacea, micrococcaceae (Dhanasekaran and Thangar, 2014). It has been suggested that the presence of bacteria in the haemolymph is indicative of septicemia and a common sequelae to stress (Lightner, 1977, 1988 in Gomez-Gil *et al*., 1998). According to Hubert *et al.,* (2015), the mite could be reservoirs of the pathogenic bacteria in the apicultures.

CONCLUSIONS

Through our experience, apparently the heamolymph of the healthy emerging worker honeybee (*Apis mellifera* L) is free for bacteria. However, the bacterial contamination enable to be transmitted into honeybee heamocoel are: *Bacillus licheniformis* (4 strains), *Bacillus mycoide* (1strain), *Bacillus coagulans* (1strain), *Brevibacillus chohinensis* (1strain), *Aeromonas hydrophila* (1 strain) and *Pantoa* sp (1 strain). The knowledge of these bacteria microflora opens up a new perspective for integrated control of this parasite, which decimates bee colonies yearly.

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Olga SARBU, Liliana CIMPOIES [1]

POVERTY REDUCTION IN RURAL AREAS THROUGH AGRICULTURAL DEVELOPMENT: EVIDENCE FROM MOLDOVA

SUMMARY

Agriculture is a main part of the rural economy and has an important role in ensuring incomes for rural population and poverty reduction. In Moldova, the largest share of the rural population is employed in agriculture, which contributes to about 50% of their total incomes. The aim of this research was to analyze the importance of the agricultural sector of Moldova and its contribution to poverty reduction of rural population. The paper estimated the time series from 2001-2015 provided by the National Bureau of Statistics. Also, the data of the General Agricultural Census (NBS, 2011) and the World Bank Report on poverty reduction (2016) were used. Nowadays, agricultural sector, despite the registered decline has an important role in the economic development of Moldova. The agricultural sector registered a decline of its share in GDP from 30% in 2005 to 12% in 2015. Also, the employment in the agricultural sector diminished from 50% in 2001 to 28% in 2015. A boost in investments in the agricultural sector would allow an alleviation in rural poverty and an increase in the population standards of living. But the problem in attracting investments is related to the fact that the large corporate farms prevail other small subsistence households which manage 60% and 40% from the useable agricultural area.

Keywords: agricultural development, households, poverty, rural areas, Moldova.

INTRODUCTION

The modern agriculture is highlighted through its dimension and potential impact at the current stage. Its multifunctional character confirms that agriculture does not represent only food production. It has tangencies with the environment, being in a direct and obvious relationship with rural areas which besides the important economic aspect assigns it a social and environmental role. Its main production function has undergone essential changes and nowadays is stressed on the safety and diversity of food products. Moreover, the 21st century generated new challenges for agriculture as price volatility, the increase of climate variability or rural poverty. The main aims of the modern agriculture are to ensure the society with competitive and healthy food at affordable prices, to satisfy the increasing needs of population both in quantity and quality, to meet the export requirements (Salasan, 2010).

[1]Olga Sarbu, (corresponding author: o.sarbu@uasm.md), Liliana Cimpoies; Department of General Economics, State Agrarian University of Moldova, Chisinau, Republic of Moldova
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The subsistence means and incomes of poor families from rural areas of Moldova mainly relies on natural resources and environment. Poor families are weak in facing such phenomena as drought, floods and climate change. Agriculture has a key role for the contribution to national income and welfare of developing countries. Thus, the welfare of poor population and development of rural areas depends on the quality and productivity of the environment (Salasan, 2009).

Agriculture is one of the most vulnerable sectors of the Moldovan economy and has a share of 12% in country's GDP (according to 2015 data) and about 60% of total area. The agriculture is determined by factors as: the increase of share of subsistence agriculture versus commercial agriculture, lack of efficiency of agricultural subsidizing system, lack of investments, excessive fragmentation of agricultural land, destroyed irrigation system. All the above mentioned contributed to the reduction of gross agricultural output and the migration of rural population to urban areas or abroad.

MATERIAL AND METHODS

This research analyses the contribution of the agricultural sector to poverty alleviation of population from rural areas of Moldova. Also, it assesses the contribution of the agriculture through the following economic indicators: the share of employed population in agriculture; the share of agricultural land in the total land fund; the share of agriculture in value added; employed population to 100 hectares of agricultural land; labor productivity per person employed in agriculture; GDP created by a person employed in agriculture.

For the assessment and analysis of given indicators was used data provided by the National Bureau of Statistics (NBS) which reveals the evolution of human resources used in agriculture, agricultural land, Gross Agricultural Output (GAO) and Gross Domestic Product (GDP) in agriculture (NBS, 2016).

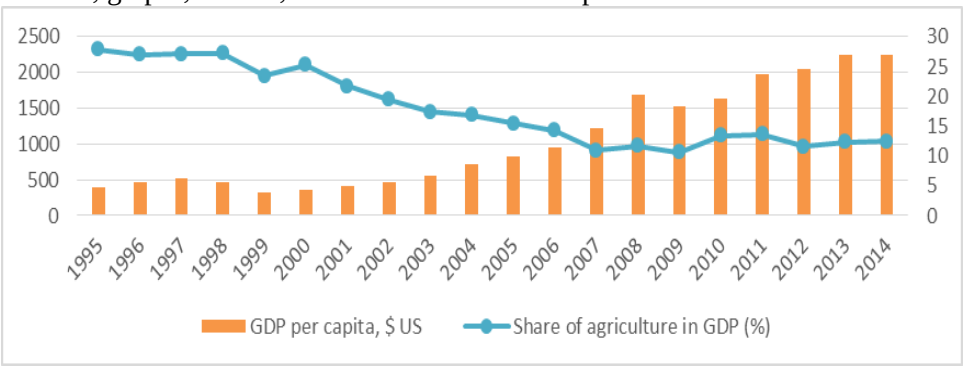
RESULTS AND DISCUSSION

The rural economy of Moldova is mainly dominated by agriculture, due to this fact poorly integrated in the market economy. Agriculture is a priority branch, which should harmoniously integrate into the economy and to increase its role as a factor of grounding and design based on the principles of free market (Stratan, 2007). Economic and social progress are closely related to the level of agricultural development and its capacity to satisfy the vital needs of population and processing industry with raw materials.

Despite the diminishing trend in the share of agriculture in GDP (from 30% to 12%) the contribution of agriculture to be much higher than its direct share in GDP. Agricultural important contribution is related to the high share in GDP of food industry, beverages and tobacco (with 24% and 10%). Also about one third of population is employed in agriculture.

Moreover, more than half of the population lives and works in rural areas. Also, a person employed in agriculture feeds on average 2.5 persons from urban

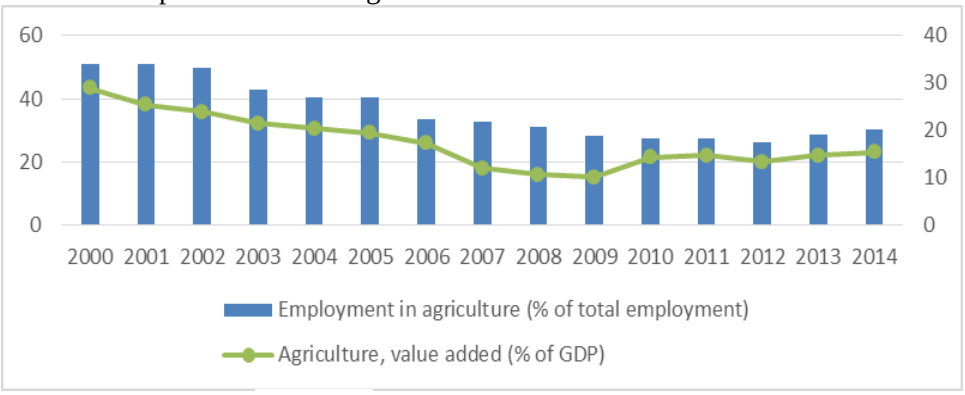
areas. In other European countries, the ration between one person employed in agriculture and employed in other sectors is 1 to 15, while in Moldova this ratio is 1 to 1.5 persons. The main agricultural products include: fruits, vegetables, tobacco, grapes, cereals, sunflower and livestock products.



*Source: based on the NBS data, 2016

Figure 1. Share of agriculture in GDP and GDP per capita in Moldova

Despite the fact in the last years the share of agriculture in GDP decreased to 13% (2014), still there are 30.5% persons employed in this sector. After the recession from 2012 the country's economy entered a period of resurrection. The Gross Domestic Product in 2013 was of $7.687b and $7.9b in 2014, with 8.9% and 7.9% more compared to 2012. The share of agriculture in Moldova's GDP is much higher than its share in the world which is of 3-3.5% and 6-7 times higher than the European Union average.



*Source: based on NBS data, 2016

Figure 2. Share of agriculture in total employment and GDP in Moldova

Moldova's agriculture has the largest share in the national value-added comparative to other Central and Eastern European countries, despite the lowest productivity level in the field (The World Bank, 2016). This is due to the high share of the agricultural sector in national GDP and the high share of population employed in agriculture. The agricultural sector has substantially potential for

growth but not enough exploited. Thus, restructuring and further sustainable development of the rural economy is required for Moldova's economic development.

The contribution of the agricultural sector at the value added was about 15% in 2013, twice more that its level in 2008. This increase was due to the stable growth of agricultural production value, except 2012 which was an extremely unfavorable for agriculture (droughts in autumn and late spring frosts). Because of its high contribution to the value added, it also had an important role in poverty reduction in rural areas.

Moldova's capacity to poverty reduction in rural areas can be assessed through the indicators presented in Table 1.

Table 1. The role of agriculture to poverty reduction in Moldova

Indicators	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Area of agricultural land per person, hectares	0,54	0,55	0,55	0,57	0,56	0,56	0,56	0,57	0,57	0,57
Employed population in economy to 1000 persons, persons	350	348	350	332	319	329	322	330	333	339
Employed population to 100 hectares of agricultural land, persons	64	63	63	60	57	58	57	58	59	59
Share of employed population in agriculture, %	33,6	32,8	31,1	28,2	27,5	27,5	26,4	28,8	30,5	31,7
Share of agricultural land, %	57,7	58,5	58,5	58,6	59,3	59,4	59,4	59,5	59,8	59,9
Labor productivity of one person employed in agriculture, $ US	2522	2770	4078	3237	5191	5974	5450	5243	4835	3621
Productivity of one hectare of agricultural land, $ US	545	583	802	545	814	962	822	905	862	683
GDP generated by one person employed in agriculture, $ US	1189	1148	1365	1226	2255	2661	2614	2795	2584	2578

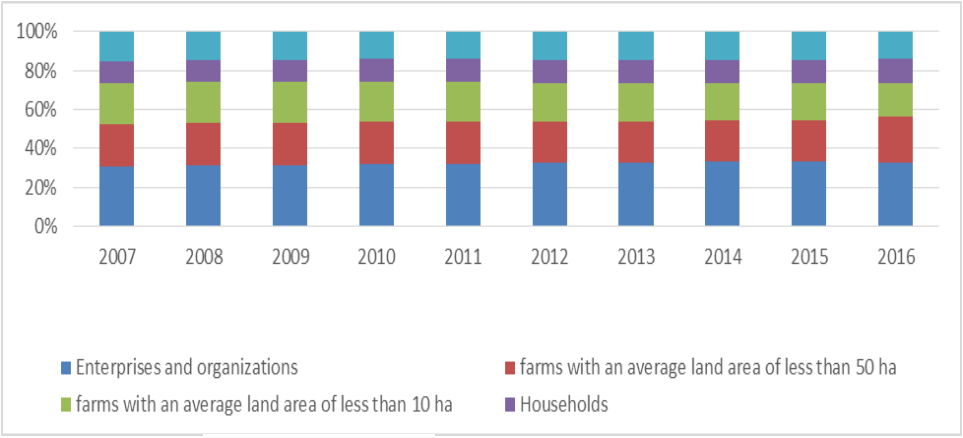
*Source: based on the NBS data, 2014-2016

The area of agricultural land per person during the examined time series remains unchanged at a level of about 0.57 hectares. The population employed in economy to 1000 persons has a diminishing tendency from 350 persons in 2006 to 239 persons in 2015 (about 3%). Also, the population employed in economy to 100 hectares of agricultural land decreased with 7% in 2015 compared to 2006. This was due to the decrease of the share of population employed in agriculture from 33.6% in 2006 to 31.7% in 2015. The share of agricultural land had minor changes with a low increasing tendency.

Table 2. Evolution of employed population from rural areas of Moldova, by economic activities, %

Economic activities	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Total employed population	100	100	100	100	100	100	100	100	100	100
from which:										
Agriculture, forestry and fishing	58,0	54,6	58,0	54,6	48,9	49,3	48,2	51,0	53,3	55,3
Manufacturing	8,0	8,3	8,0	8,3	9,06	9,4	9,1	8,6	8,5	8,4
Construction	3,0	4,8	3,0	4,8	5,26	4,9	5,4	5,3	5,0	4,5
Wholesale and retail trade	7,0	8,0	7,0	8,0	9,62	9,9	9,8	9,4	8,8	7,8
Transportation and storage	3,0	3,2	3,0	3,2	3,19	3,1	3,6	3,5	3,3	3,4
Public administration and defense; compulsory social security	18,0	18,1	18,0	18,1	20,9	20,2	20,8	18,4	17,4	17,2
Others	3,0	3,0	3,0	3,0	3,07	3,2	3,1	3,8	3,7	3,4

Source: based on NBS data, 2014-2016



*Source: based on NBS data, 2014-2016

Figure 3. Structure of agriucltural land by categories of owners (in %)

The decrease in labor productivity of a person employed in agriculture and the productivity of one hectare of agricultural land was mainly due to unfavorable weather conditions in the last period. The increase in GDP generated by one person employed in agriculture from $1189 in 2006 to $2578 in 2016 indicates to the ability of agriculture to contribute to poverty reduction in rural areas (Prisacaru, 2011).

In the last period was observed a great change in the labor force employment mostly opposite to the agricultural sector, which do not reflect the growth in other sectors; but most probably the expansion of subsistence agriculture.

Since 2006 the share of employed people in agriculture diminished from 58 to 55% in part due to immigration of the labor force from rural areas (Table 2). The employment in other sectors was lowered as well, except from trade and transportation which lightly increased. In the same time, the share of persons employed in agriculture with low intensity (less than 20 hours per week) increased constantly from 13 to 24%. This agricultural work is often carried by landowners and often is not officially considered as employment. The employment diversification by types of economic activities can made incentives for rural economy, which can determine an increase in the employed rural population. The potential of agriculture to reduce poverty is influenced by the structure and use of land. The small farms have a larger share of manual work, thus increasing the land productivity can contribute to poverty reduction.

Almost half of the agricultural land is used by farms with an average land area of less than 10 hectares, and households (Figure 3). They ensure the production of about 40% of cereals and leguminous crops, 30% of sunflower, over 80% of potatoes and vegetables and over 95% of melons and gourds, about 50% of fruits and vegetables, and over 75% of grapes.

Table 3. Dynamics of population's disposable income in rural areas of Moldova, by types of activities

Sources of disposable income	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Disposable income – total, $	56,1	77,6	94,9	76,3	86,8	101,3	103,0	107,7	96,4	84,3
By sources, in %										
-salaries	26,1	26,6	28,0	30,6	28,9	30,6	28,6	28,3	28,4	27,8
-individual agricultural activity	34,5	28,4	20,7	18,0	19,1	19,6	18,8	17,8	18,1	17,5
-income from individual nonagricultural activity	5,2	4,4	6,1	5,7	5,0	5,6	5,8	6,1	5,9	6,3
-income from ownership	0,0	0,1	0,1	0,0	0,1	0,1	0,1	0,1	0,0	0,0
-social benefits	14,8	15,3	16,3	20,6	21,2	21,3	22,5	21,3	21,2	22,0
-other incomes	19,4	25,3	28,7	25,1	25,7	22,8	24,2	26,3	26,4	26,3
from which remittances	15,8	22,2	25,9	22,0	22,8	19,7	20,8	22,9	23,4	23,4
Share of incomes in disposable income, %	33,7	27,7	20,9	19,4	19,8	19,9	19,4	17,7	17,2	17,0

Source: based on the data from National Bureau of Statistics, Aspects of the standard of living of population in 2007-2016

According to the final results of the General Agricultural Census (GAC) 2011, in Moldova are 902210 agricultural holdings, from which 99.6% are agricultural holdings without juridical status and 0.4% are agricultural holdings with juridical status.

From the total amount of holdings, 848637 are active holdings (that uses agricultural land and/or grows livestock and/or poultry) and 53577 were registered and temporarily not active holdings. Agricultural holdings own 2243540.02 hectares, from which the utilized agricultural area (UAA) is of 1940135.56 hectares, distributed to 846981 holdings. The average size of UAA per holding is of 2.29 hectares. The size is close to the 49% of the EU-27 agricultural holdings, which during the 2010 census had and an average size of less than 2 hectares. According to the census results, the UAA per capita is of 0.54 hectares, also near the EU-27 average (0.3 hectares UAA/capita). From total area about 57% is used in full ownership of the land by 97.2% of holdings, about 25% is fully leased and used by 0.3% of holdings and 15% is used by 1.4% of holdings under mixed land ownership (both ownership and lease). The other 3% of the area is used by 1.1% of holdings under other type of land ownership.

The UAA distribution is not homogenous according to the size classes of total area. From the Census results, the highest share of holdings is grouped under the smallest land size classes. Thus, 71% of agricultural holdings have less than one hectare, but the utilized area is 10.1 of total UAA. Holdings with 1-5 hectares (27%) utilizes 19.3% of total UAA. On the other side are the 0.3% of agricultural holdings with large sizes (at least 100 hectares) and utilizes 63.4% of the UAA. Almost 73% of total UAA is arable land, which is above the EU-27 average (60%) (NBS, 2011).

As income source agriculture represents a main source for 60% of population in Moldova.

Disposable income of population from rural areas registered a decreasing tendency (Table 3). The highest incomes are from salaries (28%). Incomes from individual agricultural activity decreased from 34.8% in 2006 to 17.5% in 2015. The incomes from remittances are 23.4% from total population incomes. According to a recent research, about 10% from remittances are directed to investments in agricultural holdings, for purchasing new agricultural land, buildings or machinery.

Due to lack of working places and the increased rate in inactivity, the share of labor incomes was mostly from non-agricultural sector through an increase in salaries. The fast increase in incomes from non-agricultural compared to agricultural activities lead most of employed agricultural workers among the 40% of country's poor population. Agricultural incomes were affected by various fluctuations during the analyzed time series which undermined their contribution to the increase of living conditions in rural areas. Thus, incomes diversification is viewed as a source of poverty reduction in rural areas.

CONCLUSIONS

The agriculture can contribute essentially to poverty reduction in rural areas of Moldova particularly by collaborating with other interested structures. As result of the GDP growth per capita, the role of agriculture is decreasing both for the economy and poor people from rural areas. In the same time, the development of non-agricultural activities in rural areas could contribute to poverty alleviation.

In order to increase the contribution of agriculture to poverty reduction in Moldova is needed: to improve the situation of poor population that lives in villages, to increase the competitiveness and productivity of small households, to improve the price stimulations, to increase public investments, to diversify the income sources through developing non-agricultural activities, to increase the access to financial resources and to reduce the influence of uninsured risks, to develop and apply innovations, to increase the sustainability of agriculture and to transform it in a provider of ecological services..

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Masoumeh FOROUZANI, Gholamreza MERDASI,
Zeinab NARGESI, Ziba BAKHTIARI [1]

FACTORS AFFECTING ATTITUDE OF FARMERS TOWARD ORGANIC FARMING IN KHUZESTAN, IRAN

SUMMARY

Organic farming is assumed to have the capacity to help reduction of negative economic, social and environmental impacts of green revolution, by supporting small scale farmers, meeting consumers' needs, and decrease in using chemical inputs. In line with this, during 2014-2015, a survey study was undertaken to investigate the factors affect farmers' attitudes towards organic farming. A random sample of 121 farmers was selected from west part of Iran. A multi-scale questionnaire to measure variables of general attitude toward organic farming, knowledge, perception toward economic, ecological and social goals of organic farming, perceived barriers and benefits of organic farming, and information channels, was administered to collect data. Analyzing data revealed that none of the respondents manages their farms based on organic farming style. They even do not perform soil test to learn about optimal consumption limit of fertilizers. They use higher rate of urea consumption than the optimal limit of 50 kg/ha advised by experts. Although, respondents' knowledge and attitude toward organic farming were relatively moderate (2.38 and 2.52 out of 5, respectively), their perception towards organic farming barriers (3.48 out of 5) and social goals (3.6 out of 5) showed a better status. They also highly perceived benefits, economic and ecological goals of organic farming. At last, the study concluded that to plan for changing farmers' attitude to cultivate based on organic farming a mixture of significant predictor factors including perception about ecological goals (β=0.55), perceived barriers (β=-0.54), knowledge (β=0.30) and social goals (β=0.25) must be taken into attention.

Keywords: Organic farming, Attitude, Perceived barriers, Perceived benefits, Iran.

INTRODUCTION

Historically, concerns about food have focused on its shortage and the lack of security of its supply. But, there are questions around the safety of food in light of the pesticide residues, the environmental sustainability of how it is produced, the ethical treatment of producers, the landscape agriculture creates and the treatment of animals within farming (Reed and Holt, 2006).

[1]Masoumeh Forouzani (corresponding author: m.forouzani@ramin.ac.ir), Gholamreza Merdasi, Zeinab Nargesi, Ziba Bakhtiari; Department of Agricultural Extension and Education, Ramin Agriculture and Natural Resources University of Khuzestan, Mollasani, Ahwaz, IRAN
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Recently, agriculture has been understood as more than just about producing food. It is also perceived as serving important ecological and social functions that sustain the viability of the natural resource base and the vitality of the rural life (Kroma, 2006). From the Green Revolution, there were a minority of those who were often clustered around the early organic farming movement; they questioned the reliance on chemical technologies. As this critique became louder, the organic farming movement came to prominence (Reed and Holt, 2006). Organic producers rely on resource-conserving management practices based on recycling of crop residues through green manures, composting, and using N-fixing plants to drive the multiple functions they perceive an agricultural system should serve (Kroma, 2006). It combines modern scientific research with traditional farming techniques in a sustainable, efficient farming system. By working with natural processes and making use of locally available possessions, farmers can build up the fertility and productivity of their farms while avoiding dependence on expensive external inputs (Mahmoudi and Mahdavi Damghani, 2009). Organic producers place priority on natural processes for maintaining soil fertility, soil conservation and soil health, recognizing them as critical determinants of a viable production system. Such systems adhere to minimum use of synthetic external inputs (Kroma, 2006). While, the most commonly used argument in support of organic farming is its positive environmental effects (Häring *et al*., 2001), organic farming, based on specific and precise production standards, aims to achieve an optimal farming ecosystem which simultaneously meets ecological, economic and social aspects of sustainability.

Just recently, food scares have led to a boom in demand for organic products and have increased policy makers' awareness of the potential benefits of organic farming (Häring *et al*., 2001). The demand for organic products is directly linked to growing value orientations toward food products grown under chemical-free management conditions with guarantees of natural flavors and little or no harmful residues (Pugliese, 2001).

Organic agriculture in Iran started within universities, and is taught in specific courses and lectures. Parallel to the interest for organic agriculture within the universities, the market began to develop. Certified organic products in Iran were first documented in 1999, when an orchard with roses for extracting essential oils in Kerman province was converted to organic. In 2006, another company in Fars Province was recorded to have exported organic pomegranate, figs, dates, and medicinal herbs to the European Union, and the organic market has been growing rapidly (Kledal *et al*., 2012). Although, there is a growing interest in sustainable agriculture among producers, the number of farmers shifting from conventional farming to organic forms is almost small. The area under organic production has decreased during recent years; as the area under organic production during 2011 to 2014 has declined from 43332 to 11601 hectares (Sharifi Moghaddam, 2016). However, this grossly underestimates the area under organic production, as only certified farms enter into the national statistics databases. Some products from the remote areas, for example

mountainous regions, are completely managed according to organic principles, have not been regarded as organic in the present context of organic production (Mahmoudi and Mahdavi Damghani, 2009). Overall, by taking into consideration the natural organic arena in mountainous places, the area under organic production can be estimated as 34451 hectares (Sharifi Moghaddam, 2016). Famous Iranian products are significant in organic production, including products such as pistachio, rose, dates, and pomegranate (Kledal *et al*., 2012).

There are several reasons to argue that there is a good capacity in Iranian field crop cultivation for making the transition to organic agriculture. Cultural studies have shown that Iranians always were interested in traditional products originating from the villages. Hence, organic products are favored because they are considered free from toxic chemicals, additives, artificial flavorings and colorings, preservatives, and are perceived as having a higher quality (Mahmoudi and Mahdavi Damghani, 2009). The organic sector of Iran resembles the conventional farm sector, but with a much higher concentration of very small farms (Kledal *et al*., 2012). Despite many advantages of organic agriculture, the results of several case studies show that its adoption rate is still very low among farmers (Soltani *et al*., 2014). Shifting from a conventional agriculture to an organic system comprises a risky change in perception of farmers in terms of knowledge, practice and, management skills. These uncertainties are critical factors affecting the rate of farmer transitions even though emerging evidence suggests there may be an increasing orientation toward agriculture grounded in sustainability principles among a much broader community of farmers (Kroma, 2006). Therefore, what makes it difficult for policy makers and practitioners to promote organic farming in Iran is that Iranian farmers' motives and challenges towards adopting organic production are still unclear. In addition, the few present studies have mostly considered experts' attitudes towards the dissemination of organic agriculture, not those of farmers (Soltani *et al*., 2014).

In line with this, a study was undertaken to investigate the factors affect farmers' attitudes towards organic farming.

MATERIAL AND METHODS

This survey study was conducted among farmers from September 2014 to January 2015. A sample of 121 farmers was selected through simple random sampling manner out of all farmers living in Shushtar County, west part of Iran. A questionnaire consisted of several scales to measure variables of general attitude toward organic farming, knowledge, perception toward economic, ecological and social goals of organic farming, perceived barriers and benefits of organic farming, as well as information channels, was administered to collect data. The questions were scored on a 1–5 point scale (very low, low, moderate, high, and very high). The scale reliability of the questionnaire was confirmed by computing Cronbach's alpha coefficient. All scales indicated an acceptable reliability coefficient, 0.68–0.80. Moreover, the questionnaire's validity was approved by a panel of experts. Afterwards, the study data was collected through

a face-to-face interview which conducted among our sample. All farmers were given the right to refuse to participate. There were a few farmers were not interested to answer the questionnaire, and so they were replaced by interested ones.

RESULTS AND DISCUSSION

Demographic variables showed that all the farmers participated in the study were male. Respondents were aged from 19 to 66, and had a mean age of 35.7 years (SD=10.84). The educational level of the respondents was almost low: 6.6% were illiterate, 63.6% listed a high school diploma or some secondary courses, 14.01% completed some graduate courses, 9.1% hold a bachelor's degree, and just 6.6% hold a master's degree or higher. Of the 121 respondents, only 9.1% plant under a dryland farming system, 72.7% cultivated under irrigated system, and 18.2% hold a mixed system of farming in terms of irrigation. More than 75% listed only cereals as their main crop and near 20% planted just summer crops (vegetables).

Analyzing data revealed that none of the respondents manages their farms based on organic style of farming. They even do not perform soil test to learn about optimal consumption limit of chemical fertilizers. They use Urea by average of 124 kg/ha for winter (cereals) and 130 kg/ha for summer (vegetables) cultivations, indicating a higher rate of usage than the optimal limit of 50 kg/ha which is advised by experts. There were also a few farmers who consumed a maximum amount of 300 kg/ha in contrast to those who consumed a minimum amount of 60 kg/ha of chemical fertilizer in their farms. A same trend was observed for pesticide consumption among farmers, indicating an average of 2.43 liter per hectare for cereals and 4.23 liter per hectare for vegetables. The maximum and minimum usage of pesticides among respondent farmers was 12 and 0.2 lit/ha, respectively. This shows that the fertilizer and pesticide consumption for producing crops amounts to about three times the allowable rate.

Examination of the participants' responds indicated that the Internet and the programs provided by the mass media i.e. television and newspaper were their main information source to learn about organic farming. In their view, organic products labels, markets, advise from other farmers, and educational programs hold by extension services placed at the other priorities, respectively.

The descriptive results (Table 1) revealed that the mean scores of knowledge and general attitude toward organic farming were, respectively, 16.66 out of 35 (SD=1.02) and 27.78 out of 55 (SD=5.36). Our respondents showed a relatively moderate knowledge and attitude regarding organic farming. In their views, organic farming is more rational, profitable, and pleasurable. According to Soltani et al. (2014), those Iranian farmers more likely to adopt organic agriculture are those who have a more positive attitude towards organic agriculture.

The mean score for perceived ecological goals of organic farming was 17.36 out of 20 (SD=1.64), suggesting that in the farmers' view, organic farming

contributed to viability of the natural resources, protecting wild life, improving soil fertility and increasing biodiversity. The perceived economic goals of organic farming among our sample (Mean=19.95 out of 25, SD=3.84) was relatively high. Our respondents believed that organic farming is capable to support small scale farmers by reducing production cost, providing a steady income source and economic stability. In this respect, Mahmoudi and Mahdavi Damghani (2009) have persisted that the Iranian smallholders cultivate much of their land without recourse to agrochemicals, and traditional mixed farming systems remain prevalent. In small farming system which account for more than 80 percent of agricultural products of the country still ecological practices are prevalent.

In examining our respondents' perceptions regarding social goals of organic farming, the findings showed that they moderately perceived the social goals of the organic farming (Mean=25.20 out of 35, SD=3.03). They have not completely trusted to the contribution of organic farming in improving food safety and security, meeting local needs, respecting indigenous knowledge and local culture. perceived barriers had a moderate mean score, 52.17 out of 75 (SD=5.15), which shows that our respondents moderately agreed with the lack of enough knowledge to organically produce crops, incompetency of current markets to sell organic products, insufficient information about the benefits and standards of organic products, and decrease in crop yield as the main barriers to develop organic farming among farmers. Therefore, farmers are not relatively sure whether they can sell their products in the market. These findings are also confirmed by some studies carried out on the barriers of Iranian farmers to operate organic farming (Soltani et al., 2014; Maleksaeidi et al., 2009).

However, the benefits of organic farming from our respondents' perspective gained a very high score (Mean=60.05 out of 65, SD=4.03). This means that they definitely sure that organic farming can help to decline in consumption of chemical external input, reduce the chemical contamination of soil and water resources, improve water resources quality, produce safe products, and terminate the side-effects of conventional agriculture. In addition, they believed that by organic agriculture in compare to conventional type of farming, healthier and more qualified crops with better flavor are produced. According to Kroma (2006), organic agriculture relies mostly on natural inputs as opposed to external synthetic chemicals to enhance the fertility status of the soil; its proponents suggest organic agriculture has a less harmful effect on below as well as above ground biotic life, thus engendering a more bio-diverse environment. To make easier comparing the findings, all the data were converted into a scoring scale of 0 to 5. The results revealed that, while the respondents' knowledge and attitude toward organic farming were relatively moderate (2.38 and 2.52 out of 5, respectively), their perception about benefits, economic and ecological goals of organic farming implied a high average score of 4.62, 3.99 and 4.34 out of 5, respectively. However, their perception towards organic farming barriers (3.48 out of 5) and social goals (3.6 out of 5) also showed a moderate status.

Table1. Descriptive analysis of variables

Variable	Min	Max	Mean	SD	Range
Knowledge	8	13	16.66	1.02	7-35
General attitude toward organic farming	19	37	27.78	5.36	11-55
Perception about ecological goals of organic farming	13	20	17.36	1.64	4-20
Perception about economic goals of organic farming	7	23	19.95	3.84	5-25
Perception about social goals of organic farming	18	29	25.20	3.03	7-35
Perceived barriers of organic farming	39	61	52.17	5.15	15-75
Perceived benefits of organic farming	42	65	60.05	4.03	13-65

*Source: Author's elaboration based on the questionnaire survey results

To examine what factors affect farmers' attitude toward organic farming, a regression analysis was run with attitude toward organic farming as the dependent variable and variables of knowledge, perception toward economic, ecological and social goals of organic farming, perceived barriers and benefits of organic farming as the independent variables. As Table 2 indicates, perception about ecological and social goals, perceived barriers, and knowledge are the significant predictors of the attitude toward organic farming. These variables predicted about 40% of the variance of attitude toward organic farming. Perception about ecological goals (β=0.55, p <0.0001) appear to contribute most to the model, followed by perceived barriers (β=-0.54, $p < 0.0001$), knowledge (β=0.30, $p < 0.001$) and perception about social goals (β=0.25, $p < 0.02$). Other variables had no significant effect on attitude toward organic farming. Expectedly, perceived barriers is influencing negatively the attitude.

Table 2. Regression analysis of the study variables

Independent variables	B	SEB	β	Sig.
Knowledge	1.59	0.44	0.30	0.001
Perception about ecological goals of organic farming	1.81	0.31	0.55	0.0001
Perception about economic goals of organic farming	0.015	0.10	0.01	0.88
Perception about social goals of organic farming	0.46	0.19	0.25	0.02
Perceived barriers of organic farming	0.65	0.11	-0.54	0.0001
Perceived benefits of organic farming	0.024	0.12	0.02	0.84
Constant=-37.09	**F= 11.77**		**Sig. 0.0001**	
Dependent variable	R^2	R^2Adjust	R^2Change	
General attitude toward organic farming	0.39	0.38	0.39	

CONCLUSIONS

The most important principle of sustainability in any agricultural production system is its focus on increasing the capacity of the system being managed to produce desirable environmental, social as well as economic benefits. Organic farming is assumed to have the capacity to help the reduction of negative economic, social and environmental impacts of green revolution, by supporting small scale farmers, meeting consumers' needs, and decrease in using chemical external inputs. According to Mahmoudi and Mahdavi Damghani, (2009) organic agriculture will enable Iranian smallholders to achieve household food security and gain better incomes while regenerating the land, enhancing biodiversity, and supplying qualified food to local communities.

This study revealed firstly that none of the respondents manages their farms based on organic style of farming. The Internet is the most popular source of information and the preferred choice for news ahead of television, newspaper, farmers' face to face visits and extension services among farmers. While, farmers' knowledge, general attitude toward organic farming, perception about social goals and barriers to operate organic farming were relatively moderate, their perception about economic and ecological goals and benefits of organic farming implied a high to very high average score. At last, the study concluded that to plan for changing farmers' attitude to cultivate according to organic qualifications a mixture of significant factors including perception about ecological goals, perceived barriers, knowledge and social goals must be taken into attention. In contrast to other influential factors, perception about barriers is the most significant factor which affect attitude negatively. Finally, this study underlined the necessity that future efforts should concentrate on raising the awareness of all farmers and on building the capacities of Extension services to establish a knowledge system supportive of sustainable agriculture development policies generally, and organic farming policies specifically.

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Tonya GEORGIEVA, Ivanka TOROMANOVA[1]

FORMATION OF PANICLE BASIC ELEMENTS WITH TURKISH AND ITALIAN RICE CULTIVARS GROWN IN BULGARIA

SUMMARY

A two-factor field experiment was carried out in the Saedinenie region, Plovdiv municipality, Bulgaria in the period 2013 - 2015 following the split plot design with size of the reporting plot of 14 m2. Two Turkish (Osmanchik 97 and Gala) and four Italian cultivars (Lince, Cameo, Puma and Brio) were studied in the survey, grown on the larger plots (Factor A). Three foliar treatment products – Folur, Amalgerol and Litovit were set on the smaller plots (Factor B). The non-treated variant of each variety was used as control. The following basic panicle indices were studied: number of full and empty spikelet, panicle grain weight. All panicle elements were best developed in the favorable 2013 year. The Turkish cultivars formed the greatest number of grains with highest weight respectively, only under most favorable conditions (2013). The Italian cultivars, with the exception of Brio, behaved in a more adaptive manner throughout years with greater stress for the plants, especially during the flowering period (2014 and 2015). They formed grain from 2.5 to 2.9 g in 2014 and from 2.6 to 2.7 g in 2015. The application of foliar treatment products supported the plants to overcome stress and increased the grain weight.

Keywords: Rice (*Oryza sativa* L.), cultivars, panicle elements, foliar treatment products.

INTRODUCTION

According to Fageria *et al.* (1999) the main components of the rice yield are: number of panicles per unit area, number of spikelet in panicle, the fertility of the spikelet and the weight of the grain. It is considered that the number of the spikelet, usually, is one of the most volatile components, which is responsible for about 74% of the yield variations. On the other hand, Yoshida *et al.* (1976) express the opinion, that the fertility percentage of the spikelet and the grain weight, together, are responsible only for 26% of the changes in its value.

A significant effect of the genotype over the number of the grains per panicle is established by many authors. In the selection programs, targeted towards cultivate improvement, the big panicle and high

[1]Tonya Georgieva (corresponding author: tonia@au-plovdiv.bg), Ivanka Toromanova, Agricultural University – Plovdiv, BULGARIA.

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production of biomass are considered as the major selection criteria (Khush, 2001), i.e. the panicle is the major field of work for the achievement of higher yields. As contrary to the above Jennings (1979) it is believed that the characteristics of the panicle just help in the determination of the elements of the productivity. The characteristic, which generally was used up to now for description of the quality of the rice was the weight of the grains (Zakaria *et al*., 2002; Morita *et al*., 2002).

The purpose of the study is to provide a comparative characteristics of perspective Turkish and Italian rice cultivars in relation to the formation of panicle basic elements and the influence of the foliar treatment products – Folur, Amalgerol and Litovit over them.

MATERIAL AND METHODS

In the period 2013-2015 a two-factor field experiment using the split plot method was carried out in the town of Saedinenie, Plovdiv region, Bulgaria. The basic studied factor (big plots) in the experiment was the genotype. Six introduced cultivars were tested – two of Turkish origin

(Osmanchik 97 and Gala) and four of Italian origin (Cameo, Lince, Puma and Brio), whereas Osmanchik 87 was used as the standard. The tested foliar treatment products (Folur, Amalgerol and Lithovit) were applied on the small plots. A non-treated control for each cultivar served for comparison. The experiment was set after rice as a fore-crop in four repetitions, with reported size of the harvested plot of 14.85 m^2.

The statistical procession of the test data was made through SPSS V.9.0 for Microsoft Windows (1999). Variation proofs were indicated at significance level P 5%.

The meteorological conditions for the vegetation year 2013 were favourable for rice growing (Fig. 1). The average temperatures from May till October were by +0.2°C to +3.8°C higher, compared with those in the long-term period. The latter contributed to: growth and development acceleration, formation of maximum productivity and shortening the vegetation period.

The agro conditions in 2014 were completely different from those in 2013. The average daily temperature results for the first two months of the vegetation period (May – June) showed, that at this stage rice was in less favourable conditions than the previous year. In the process of tillering and reaching full maturity, the registered temperatures were comparatively close to those in the long-term period. The average monthly rainfall for the whole vegetation period in 2014 was dramatically heavier compared to the long-term period and 2013 (Fig. 2). The registered heavy rains proved to be unfavourable for rice development as they changed the regulated water regime and microclimate in the rice enclosure. The amount of rainfall was lower only in August and the registered temperatures reached 35.6°C, which did not facilitate normal rice flowering.

The climatic situation in 2015 is generally very dynamic. It is characterized by favorable conditions in the first stages of rice development (similar to 2013), but the sharp increase in rainfall in the second half of the vegetation brings it closer to the unfavorable 2014.

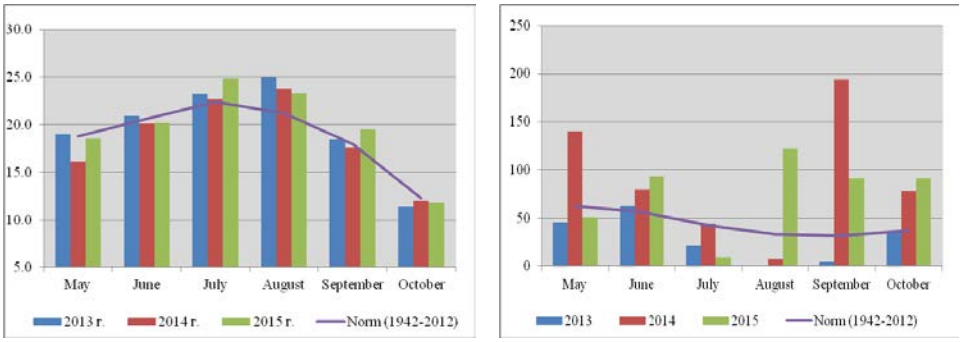

Fig. 1. Average monthly temperatures (°C) and total sum of rainfall (mm/m^2) for the research period, 2013-2015

RESULTS AND DISCUSSION

Number of full spikelet (grains) per panicle. Table 1 shows the data for number of full spikelet (grains) in a panicle, counted in the favorable year 2013. Significant differences are established between the tested cultivars. With Gala cultivar, in 2013, the highest number was formed (92.4), which exceed the control Osmanchik 97 by 14.8%. All other cultivars were categorized within the boundaries of the control. Only with Lince cultivar, which during that year showed lowest productivity, their number is proven to be the lowest.

The tested products had positive influence over that indicator – with up to 5% increase compared to the non-treated variants, but that result cannot be statistically proven.

In year 2014 (Table 1) the categorical domination of all tested cultivars over the control Osmanchik 97, which has formed only 46.5 grains per panicle, was established. The differences were from 34 to 79% with different cultivars and these differences were statistically proven. The highest number of grains were present with the Italian cultivars Lince and Cameo (83.4 and 79.1 pieces) and the Turkish cultivar Gala (83.1 pieces). The serious differences of that indicator in one less favorable year showed specific cultivar sensitivity of the tested cultivars to stress factors.

The analysis of data from year 2015 confirmed and proved analogical dependence, established in the previous year, 2014. The two Turkish cultivars were with the lowest average values - of 60.6 pieces for cultivar Osmanchik 97 up to 60.9 pieces for cultivar Gala. The cultivar with the highest yield, Cameo,

was with proven superiority in the number of grains per panicle (76.8 pieces), reaching over 26.6% compared to the control (Table 1).

The tested foliar treatment products had positive influence over this indicator during the experimental years, but the differences were within the range of 3 to 7 pieces per panicle (4,4 to 11.8%) and were not statistically proven.

Table 1. Effect of the cultivars and foliar treatment products on the number of grains per panicle for 2013, 2014 and 2015.

Factor	Variance analysis of the impact of factors on the number of grains per panicle					
	2013		2014		2015	
	Number	%	Number	%	Number	%
Cultivar						
Osmanchik 97	80,4 b	100,0	46,5 c	100,0	60,6 b	100,0
Gala	92,4 a	114,8	83,1 a	178,8	60,9 b	100,3
Linche	69,8 c	86,8	83,4 a	179,4	75,8 a	125,0
Kameo	75,0 bc	92,2	79,1 a	170,2	76,8 a	126,6
Puma	82,4 b	102,4	68,3 b	146,9	67,4 b	111,1
Brio	79,0 b	98,2	62,3 b	134,1	64,2 b	105,8
Foliar treatment product						
Control	77,1 a	100,0	66,7 a	100.0	64,1 a	100,0
Folur	80,5 a	104,4	71,9 a	107,8	67,1 a	104,7
Amalgerol	80,9 a	104,9	71,6 a	107,3	71,6 a	111,8
Litovit	81.0 a	105,0	71,5 a	107,1	67,6 a	105,6

Means followed by the same letter are not statistically different (P<0,05) by Duncan's multiple range test.

The data, presented in Table 2, were analyzed for the performance of complex analysis of the influence of all tested factors (year, cultivar and product) during the entire period of the study.

The individual influence of the year over the number of grains in the panicle corresponded to the tendency, which is accounted in the productivity. A proven higher number of grains in the panicle was formed in year 2013, where their average number was 79.8.

When considering the influence of the cultivar over the number of the grains per panicle, it was established that all of the tested cultivars surpass the control Osmanchik 97 and the differences vary up to 26%. Gala, Lince and Cameo were distinguished as cultivars with highest number of grains per panicle during the three test years (up to 78 pieces).

Analyzing the data of the influence of the tested foliar treatment products over the grains, it was established that there were no statistically proven differences, but such were observed to up to 7.8% in favor of the most effective product – Amalgerol.

Weight of the full spikelet (grains) in panicle. The most important indicator, directly related to the yield is the weight of the grains, formed in a

panicle. The observations, performed in year 2013 and the tracked influence of the cultivar proved (Table 3) that the two Turkish cultivars, Osmanchik 97 and Gala, formed the highest weight of grains in panicle and all other cultivars fall below their values. The treatment with foliar products showed an effect compared to the control cultivar, equal to 12.3-13.9% in favor of Litovit and Amalgerol and a smaller effect (3.2%) with Folur.

Table 2. Impact of main factors (variance analysis) on number of grains per panicle in rice

Number of grains per panicle (GP) in rice and Duncan's test (DT)											
	GP	DT	%		GP	DT	%		GP	DT	%
Year effect				Cultivar effect				Foliar treatment effect			
				Os. 97	62.5	c	100.0				
2013	79.8	a	100.0	Gala	78.8	a	126.0	Contr.	69.3	a	100.0
2014	70.4	b	88.2	Linche	76.3	a	122.1	Folur	73.2	a	105.6
2015	67.6	b	84.7	Kameo	77.0	a	123.1	Amal.	74.7	a	107.8
				Puma	72.7	ab	116.3	Litovit	73.4	a	105.9
				Brio	68.5	abc	109.6				

Means followed by the same letter are not statistically different (P<0,05) by Duncan's multiple range test

The cultivars Gala, Lince, Cameo and Puma had values of 2.5 g up to 2.8 g 2014 (Table 3), which significantly surpassed the control cultivar Osmanchik 97 with more than 1 g per panicle. Surpassing of the weight of the formed grains in panicle was observed with the plots, treated with Folur, Amalgerol and Litovit, but with a lower extend (2.3 to 9.5%) compared to the previous year.

For 2015, considering the similar agro-meteorological conditions with year 2014, the statistical analysis showed close tendencies. The difference was observed in the fact that Gala (2.0 g), fall in the category of Osmanchik 97. The above corresponded to our conclusions, that the Turkish cultivars perform with good productivity only in favorable year, in terms of agro-meteorological conditions, as was year 2013. The increase of the weight of the grains per panicle was also observed as a result of the usage of the products Amalgerol and Litovit.

The data for the complex influence of the factors year, cultivar and tested preparation over the weight of the grains per panicle, in the period of the three-years test are presented in Table 4.

In relation to the influence of the year over that indicator, it was statistically proven that the highest weight was observed for the cultivars in year 2013.

The data about the influence of the cultivar over the weight of the grains per panicle clearly showed that all tested cultivars surpass the control. The cultivars were most positively influenced by Amalgerol – with 12% increase, followed by Folur and Litovit – about 7%.

Table 3. Effect of the cultivars and foliar treatment products on the grain weight per panicle

Factor	Variance analysis of the impact of factors on the grain weight per panicle					
	2013		2014		2015	
	g	%	g	%	g	%
Cultivar						
Osmanchik 97	3.1 a	100.0	1.6 b	100.0	2.0 c	100.0
Gala	3.1 a	99.4	2.6 a	161.0	2.0 c	101.5
Linche	2.4 c	75.4	2.7 a	167.1	2.5 a	130.3
Kameo	2.5 c	78.3	2.8 a	170,1	2.8 a	145.6
Puma	2.8 b	87.9	2.5 a	149.4	2.3 b	117.4
Brio	2.4 c	77.0	1.6 b	95.7	2.1 b	109.2
Foliar treatment product						
Control	2.5 b	100.0	2.2 a	100.0	2.2 b	100.0
Folur	2.6 ab	103.2	2.4 a	109.5	2.3 ab	104.2
Amalgerol	2.9 a	113.9	2.3 a	105.4	2.5 a	114.4
Litovit	2.8 a	112.3	2.3 a	102.3	2.3 a	105.1

Means followed by the same letter are not statistically different (P<0,05) by Duncan's multiple range test.

Number of empty (sterile) spikelet in a panicle. The conditions, in which the panicle is formed are determinant for the achievement of the potential of the observed biometric parameters. The high percentage of empty spikelet can be due to the low solar radiation 3 weeks before and after the panicle emergence. There are also genotype differences. The cloudy and rainy weather in the period of accumulation of plastic substances has negative effect over the carbohydrates synthesis and their accumulation in the grain (Bradbrook, 2012).

The number of empty spikelet per panicle is a function, first of all, of the unfavorable agro-meteorological conditions during the blossoming period and growing of the grains.

The values accounted in year 2013 (Table 5) showed insignificant loss of the potential of the panicle due to empty spikelet (5.4 pieces). This fact is in absolute unison with the panicles rich in grains and the high yield during that year. But in years 2014 and 2015, the panicles had about 15 pieces of empty spikelet. We relate the high number to the insufficient sunshine and abundant rainfalls in the critical phases in those years.

The highest number of empty spikelet per panicle average for the period was observed with Osmanchik 97 (17.15 pieces) and Brio (15.70 pieces). The impact of the tested products is insignificant.

Table 4. Impact of main factors (variance analysis) on grain weight per panicle in rice

Grain weight per panicle (GW in g) in rice and Duncan's test (DT)											
	GW	DT	%		GW	DT	%		GW	DT	%
Year effect				Cultivar effect				Foliar treatment effect			
				Os. 97	2.1	b	100.0				
2013	2.7	a	100.0	Gala	2.6	a	122.9	Contr.	2.3	A	100.0
2014	2.4	b	88.9	Linche	2.6	a	121.4	Folur	2.5	A	107.3
2015	2.3	b	85.2	Kameo	2.7	a	127.6	Amal.	2.6	A	112.0
				Puma	2.5	a	119.0	Litovit	2.5	A	106.9
				Brio	2.5	a	118.6				

Means followed by the same letter are not statistically different (P<0,05) by Duncan's multiple range test

Table 5. Impact of main factors (variance analysis) on number of empty spikelet per panicle

Number of empty spikelet per panicle (ESN) in rice and Duncan's test (DT)											
	ESN	DT	%		ESN	DT	%		ESN	DT	%
Year effect				Cultivar effect				Foliar treatment effect			
				Os. 97	17.2	a	100.0				
2013	5.4	b	100.0	Gala	10.4	b	60.4	Contr.	11.1	A	100.0
2014	14.8	a	272.0	Linche	8.3	b	48.1	Folur	12.2	A	110.0
2015	15.0	a	275.3	Kameo	7.8	b	45.2	Amal.	11.4	A	102.1
				Puma	11.0	b	64.3	Litovit	12.2	A	109.3
				Brio	15.7	a	91.5				

Means followed by the same letter are not statistically different (P<0,05) by Duncan's multiple range test

CONCLUSIONS

The number of formed grains per panicle is within the limits from 67 to 79 pieces and is proven to be influenced by the conditions during the year. All tested cultivars surpass the control Osmanchik 97 with differences up to 26%. Gala, Lince and Cameo are distinguished as cultivars with highest number of grains (up to 78 pieces). Amalgerol is most effective and it increases the number of grtain per panicle with almost 8%.

The weight of the grains per panicle is highly influenced (with differences from 11 to 15%) by the conditions during the years. The cultivar Cameo is distinguished with a weight of the grains of 2.7 g (28% more compared to the control). Amalgerol increases the weight with 12% and Folur and Litovit – with about 7%.

The number of empty spikelet per panicle is most significantly dependent on the agro-meteorological conditions during the years and the differences can reach up to 175%. All tested cultivars (with the exception of Brio) form panicles with smaller number of empty spikelet compared to Osmanchik 97.

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Natalia YEGOROVA, Valentina BRAILKO, Irina STAVTZEVA, Irina MITROFANOVA [1]

SOME MORPHOPHYSIOLOGICAL FEATURES OF LAVANDER CULTIVAR MICROPROPAGATED *IN VITRO* BY MERISTEM CULTURE

SUMMARY

The data about some morpho-physiological peculiarities of explants of lavender (*Lavandula angustifolia* Mill.) the cultivars Stepnaya, Sineva, Vdala, Record, Rannyaya, introduced by meristems in aseptic conditions and under long-term micropropagation *in vitro* are presented. During cultured meristems on Murashige and Skoog (MS) medium with 1.0 mg L^{-1} Kinetin and 0.5 mg L^{-1} GA_3 all cultivars have formed the multiple shoot. The maximum number of microshoots at *in vitro* introduction stage was in the cultivar Sineva (5.7), and minimum – in the cultivar Rannyaya (3.9). Further, microcutting of main and additional shoots was conducted every 30-35 days. Obtained shoot segments with one node on MS medium with 1.0 mg L^{-1} Kinetin and 0.5 mg L^{-1} GA_3 were cultured. When micropropagation of lavender follow 6th subculture it was shown that the number of shoots was increased in all cultivars till 3 subculture, and then it was decreased. In the cultivar Sineva the maximal multiplication index (12.2) was noted at the third passage, and then changed from 11.5 to 7.4. The lowest multiplication index at 5-7th passages was in the cultivar Record (3.8-4.5). Analysis of water regime of obtained microshoots at 6th subculture showed that the total water content was 83-89%, and the fraction of bound and colloid-absorbed water was 40-57%. Maximum values of water-holding capacity in the cultivars Record, Rannyaya and Vdala were found. Studying the parameters of light-induction processes it has been showed that the cultivars Vdala and Sineva were characterized by maximum photosynthetic activity of leaves and shoots, and they had high viability index.

Keywords: *Lavandula angustifolia*, clonal micropropagation, multiplication index, water regime, photosynthetic activity.

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[1]Natalia Yegorova (corresponding author: yegorova.na@mail.ru), Federal State-Funded Institution of Science "The Labour Red Banner Order Nikita Botanical Gardens – National Scientific Center of RAS", Nikita, RUSSIA, Federal State-Funded Institution of Science «Research Institute of Agriculture of the Crimea», Simferopol, Crimea, Valentina Brailko, Federal State-Funded Institution of Science "The Labour Red Banner Order Nikita Botanical Gardens – National Scientific Center of RAS", Nikita, RUSSIA, Irina Stavtzeva, Federal State-Funded Institution of Science «Research Institute of Agriculture of the Crimea», Simferopol, Crimea, Irina Mitrofanova, Federal State-Funded Institution of Science "The Labour Red Banner Order Nikita Botanical Gardens – National Scientific Center of RAS", Nikita, RUSSIA

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INTRODUCTION

Lavender is one of the most widely spread essential oil, medicinal and spicy aromatic plants, which is grown in many countries of the world. In Russia and the southern regions of Europe is predominantly cultivated *Lavandula angustifolia* Mill., less often – *L. latifolia* Vill., *L. stoechas* L. and lavandin (*Lavandula x intermedia* Emeric ex Loisel) (Bochkarev and Zelentsov, 2013). Plant raw material of lavender contains essential oil and complex phenolic compounds, such as flavones, anthocyanins (Harborne and Williams, 2002). Lavender essential oil, the main components of which are linalyl acetate, linalool and geraniol, is used in the perfumery, cosmetics and food industries, in ceramic and paint production. It is used in medicine as wound healing, antispasmodic and sedative agent. Lavender is also successfully used as ornamental, honey and anti-erosion plant, cultivated on sloping lands.

Traditional methods of lavender vegetative propagation (woody or green stem cutting) do not allow to obtain high quality planting material with a high multiplication index, that can be overcome by micropropagation *in vitro*. There are data on some aspects of clonal micropropagation of *L. officinalis* (Chishti et al., 2006), *L. dentata* (Bona et al., 2011), *L. angustifolia* (Hamza et al., 2011, Yegorova, 2008), *L. viridis* (Dias et al., 2002), *L. vera* (Andrade et al., 1999), *L. pedunculata* (Zuzarte et al., 2010), lavandin (Lucchesini et al., 2006, Mitrofanova et al., 2016) and other lavender species. Most of these studies were devoted to optimization of culture media composition for growth of microshoots and their rooting. The best development of microshoots in *L. viridis* was achieved when explants cultured on the medium with BAP (Dias et al., 2002), in *L. dentate* – BAP and IBA (Echeverrigaray et al., 2005), in *L. officinalis* – BAP and IAA (Chishti et al., 2006), in *L. angustifolia* – TDZ (Hamza et al., 2011) or Kinetin and GA_3 (Yegorova, 2008). In some studies it is analyzed the influence on the efficiency of micropropagation a species or cultivars (Bona et al., 2011, Yegorova and Stavtzeva 2015), type of explant (Bona et al., 2011, Hamza et al., 2011). Biochemical and physiological features during lavender and lavandine cultivars clonal micropropagation were revealed and adaptive capacity of this two essential oil species was investigated (Mitrofanova et al., 2016, Grebennikova et al., 2017). However, many questions concerning propagation *in vitro*, especially during long-term subculture, while insufficiently were studied.

The aim of this work was to study the morphometric and physiological parameters of explant development in five *L. angustifolia* cultivars under long-term micropropagation *in vitro*.

MATERIAL AND METHODS

Five cultivars of lavender (*Lavandula angustifolia* Mill.) – Stepnaya, Sineva, Rannyaya, Vdala, Record were investigated. The initial plants were grown in field condition of Crimea foothill zone. As a primary explants meristems with one pair of leaf primordia from axillary buds were used. For micropropagation the segments of the stem with a node (5-7 mm), obtained by

microcutting of microshoots were used. Meristems were cultivated on the modified Murashige and Skoog (MS) medium with 1.0 mg L^{-1} Kinetin and 0.5 mg L^{-1} GA_3, microcuttings – with 0.5 mg L^{-1} Kinetin and 0.1 mg L^{-1} GA_3 (Yegorova, 2008). Explants in test tubes were kept at 24-26°C, 70% of relative air humidity, under 16-h photoperiod with illumination 2-3 klx. Microcuttings was conducted every 30-35 days, before which the length and number of shoots, the number of nodes (pairs of leaves) on shoot, the frequency of multiple shoot formation, callusogenesis and rhizogenesis were determined. The multiplication index was calculated as the number of microcuttings that can be obtained during one subculture. For this the number of formed on the explant shoots was multiplied on the number of nodes on the shoot. Experiments on *in vitro* culture were carried out in three replications; at each variant 20 explants were analyzed.

The total water content in leaves and its fractional composition were studied and analyzed (Lishchuk, 1991) as physiological criteria characterizing the water regime in plants. The parameters of photosynthetic activity were measured using a portable fluorometer LPT-1/CFU (Russia), which had been designed to assess the functional state of photosynthetic apparatus in various plant organs and tissues *in vitro* according to the parameters of chlorophyll fluorescence induction. In the device fluorescence is excited in the blue spectral region (470 nm), the Kautsky curve is detected, and on its basis indexes of the light energy photosynthetic transformation in the plant cell are determined. During the experiments we recorded such components of fluorescence induction kinetics as initial fluorescence level (F_0), maximum (F_m) and stationary (F_{st}) fluorescence after the light adaptation. Based on the data obtained, the viability index and photosynthetic activity were calculated (Korneev, 2002, Budagovsky et al., 2002) that allows to evaluate the functional state of photosynthetic tissues and the whole organism (Budagovsky et al., 2002). The experiments were performed in 3 biological and 3 analytical replications. For statistic description of experimental results methods of statistical analyses were used (STATISTICA 6.0 Software).

RESULTS AND DISCUSSION

When isolated meristems of lavender cultivars have been introduced into *in vitro* culture, the development of main microshoot was started in a week of cultivation. Two weeks later, adventive buds were appeared, from which shoots were regenerated. The frequency of multiple shoot formation in all cultivars was quite high and reached a maximum in the cultivar Sineva (94.1%). The number of microshoots depended on the genotype and varied from 3.9 (the cultivar Rannyaya) to 5.7 pcs / explant (the cultivar Sineva). At the stage of the meristems *in vitro* introduction, in addition to the development of shoots, the formation of 1-2 small roots with a frequency of up to 12.5% was observed in the cultivars Vdala and Record. At the base of microshoots with frequency of 6.5-17.8% in the cultivars Rannyaya and Vdala a small dense callus was developed. It should be noted that the induction of callusogenesis is undesirable during micropropagation because the probability of somaclonal variants occurrence,

therefore the emerging callus was removed by subculturing. Some authors when cultured lavender buds or nodal segments also revealed callus formation (Dias et al., 2002, Zuzarte et al., 2010, Bona et al., 2011).

Shoots, obtained from meristems *in vitro*, for further micropropagation were divided into single-node microcuttings and transferred on Murashige and Skoog medium with Kinetin and GA_3. At this stage (micropropagation proper), one or two shoots (up to 20-30 mm long with 4-5 nodes) from the axillary buds of the microcuttings and up to 6-10 additional shoots were developed. On average, from the microcutting, depending on the cultivar and number of subcultures, were developed from 1.6 to 3.5 shoots with 1.9-3.6 pairs of leaves (nodes). Our results indicate the possibility of using several methods for lavender multiplication – microshoots cutting, induction of axillary and adventitious shoots. This allows to increase the multiplication index. In studied cultivars at this stage the rare cases of callus or root formation, as well as development of hyperhydrated shoots, were noted. The exception was the cultivar Sineva, which formed the greatest number of shoots, some of them showed signs of vitrification. In some studies, concerning the lavender micropropagation, the formation of hyperhydrated shoots also have been shown. Frequency of such shoots regeneration depended on the concentration of growth regulators (Andrade et al., 1999) or agar (Lucchesini et al., 2006). Hamza et al. (2011) noted that in *L. angustifolia* the frequency of vitrification was reduced with increasing sucrose content and under using ½-¼ strength culture medium.

An important question at the second stage of micropropagation is the study of influence of subcultures number on the development of meristem cultures. This is necessary to obtain a multiply microplants during long time. Study of the lavender micropropagation during six subcultures showed the variability of some morphometric parameters and the multiplication index, which are depended on the duration of cultivation. By third passage the number of shoots and the number of its nodes were increased, which led to increase the multiplication index in all studied cultivars (Figure 1). The highest multiplication index had the cultivars Sineva and Rannyaya. After that, at 5-6th subcultures a decrease of the multiplication index (almost to the level of the first passage) and the stabilization of this parameter were observed. The most active regeneration of adventitious shoots was noted in the cultivar Sineva, which had the maximum multiplication index (up to 12.2).

The cultivars Vdala and Rannyaya in the 3-4th passages also showed a high micropropagation capacity. It should be noted that, in contrast to other studied cultivars, in the cultivar Sineva the multiplication index at 5-6th subculture was significantly higher than in the first one. The obtained data indicate the possibility of prolonged clonal micropropagation of *L. angustifolia* cultivars *in vitro*. When analyzing the effect of the duration of culture on the micropropagation of certain essential oil plants, it was found that in geranium the multiplication index during two years have not changed. At the same time in sage and fennel during the first three passages the multiplication index have not

changed significantly, and then gradually have decreased (Yegorova et al., 2013). The study of essential oil rose cultivars during 9 subculture shown the increase of multiplication index at 3-4th passages (Yegorova et al., 2015).

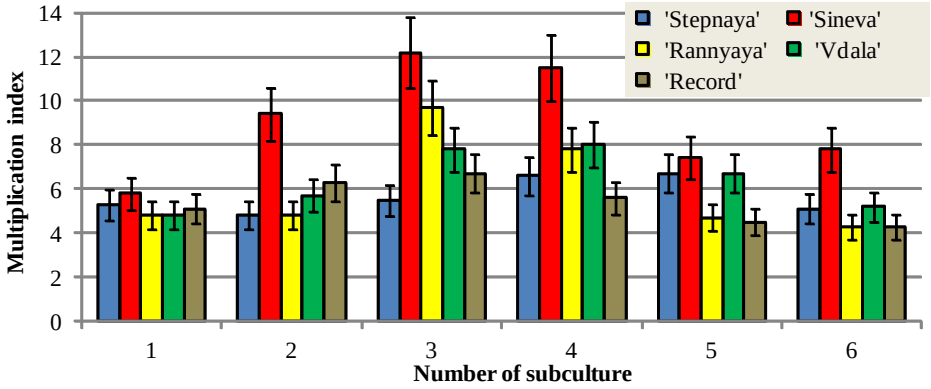

Figure 1. Influence of subculture number and cultivar on the lavender *in vitro* multiplication index

The ability to regulate water regime under aseptic conditions is one of the characteristics of their high adaptive capacity in the subsequent stages of *in vivo* and *ex situ* cultivation. Seeing some xeromorphic features in the organization of *L. angustifolia* vegetative organs *ex situ* (Rabotyagov et al., 2014) and *in vitro* (Brailko et al., 2017), we suppose the presence of genetic potential for the implementation of water stress protective mechanisms at all stages of biotechnology process of micropropagation.

Microshoots of different cultivars obtained *in vitro* at the sixth subculture were used for physiological investigations. Lavender cultivars studied *in vitro* were highly watered, the total water content was 83-89%. Wherein, there were no significant differences among the studied cultivars on this parameter. The part of bound water was 40-57% of the total water content. The maximum osmotic and colloid-bound water was noticed in the cultivars Record (56.65%), Rannyaya (54.51%) and Vdala (47.64%). The data obtained are corresponded with the results of previous studies in some *L. angustifolia* and *L. hybrida* Rev. cultivars carried out during the first passage (2 months) on the modified MS medium with 0.3 mg L^{-1} Kinetin, 0.025 mg L^{-1} NAA and 0.25 mg L^{-1} GA_3 (Brailko et al., 2017; Mitrofanova et al., 2016).

Studies of the structural rearrangements dynamics in living objects are a promising but very complex problem in experimental biology. Especially relevant is the use of non-invasive methods for recording activity of the photosynthesis process, which allows the cultivars to remain vital and continue to monitor their functional state continuously. Some indexes of lavender assimilation capacity *in vitro* were investigated. The revealed features of light-induction processes indicated a high photosynthetic activity in leaves and shoots of regenerants. Thus, the relative photosynthetic activity $(F_m-F_{st})/F_m$ varied from

0.51 ± 0.04 to 0.68 ± 0.04 a.u. However, the cultivars Vdala and Sineva were characterized with the maximum values of this index, and in the cultivar Rannyaya it was minimal. Viability index (F_m/F_{st}) was 1.38-2.71 and it was maximum in the cultivars Vdala (2.71±0.05) and Sineva (2.56±0.18). In the cultivar Stepnaya we noticed minor abnormalities in the functioning of light-harvesting complexes: a significant part of the light energy was reflected, the maximum fluorescence was 117 a.u. Markers of lavender microshoots functional state determined by the method of chlorophyll fluorescence induction demonstrated the absence of photoinhibition and normal functioning of photosystems both at the level of light-harvesting complexes (the exception was the cultivar Stepnaya) and at the moment of electron donors oxidation in the photosystem II reaction center.

CONCLUSIONS

The morphophysiological peculiarities of explants in the process of 6 subcultures in micropropagation of five lavender cultivars have been identified. An increase of the multiplication index by third subculture was shown. The maximum ability for adventitious shoot formation and a high multiplication index was found in the cultivar Sineva.

Significant water retention capacity due to the bound water fraction was found under the high values of the total water content in leaf tissues of microshoots. Normal functional state of assimilating tissues in microshoots was demonstrated. The high photosynthetic activity of the leaves and the viability of the cultivars Vdala and Sineva have been identified, which corresponds to their best regenerative capacity during prolonged microcutting and a higher multiplication index. This is especially true for the cultivar Sineva, which was distinguished among the studied genotypes, both during the stage of meristem introducing and during six subcultures *in vitro*.

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Jasmina BALIJAGIĆ, Jovan CRNOBARAC, Miodrag JOVANČEVIĆ, Irfan MUJANOVIĆ [1]

THE EFFECT OF VARIETY AND PLANT DENSITY ON POT MARIGOLD FLOWER YIELD (*Calendula officinalis* L.) IN AGRO-ECOLOGICAL CONDITIONS OF BIJELO POLJE

SUMMARY

Collecting medicinal plants from their natural habitats cannot meet the needs of the increased demand for natural medicinal raw materials in the global market, which caused the expansion of medicinal plants cultivation. This paper deals with the influence of the three varieties of pot marigold; "Plamen Plus", "Bački Petrovac" and "Orange King" in different sowing density on pot marigold yield.

By studying the effects of the variety and plant density on a total yield of fresh inflorescence of marigold, it was found out that variety has a statistically significant effect on yield. The variety "Plamen plus" has achieved the higher yield (5724 kg ha^{-1}) comparing with the other two varieties which did not differ significantly, although the lowest yield was obtained by the variety "Orange King" (3243 kg ha^{-1}).

Keywords: *Calendula officinalis*, pot marigold, variety, yield

INTRODUCTION

Collecting medicinal plants from their natural habitats cannot meet the needs of increased demand for natural medicinal raw materials on the world market. Today the efforts are made to meet these needs by medicinal plants cultivation/plantation coupled with field inspection, in order to obtain healthy, not pesticide contaminated raw material, uniform quality, sufficient quantity and meet the agreed deadlines (Mrđa et al., 2007; Buha, 2012).

Pot marigold (*Calendula officinalis L.)* is an annual or, less commonly, biennial herbaceous plant. It is successfully cultivated in areas with moderately warm and moderately humid climate. The sowing forecast is, in fact, the forecast of the date when the sowing layer of soil will be warmed up to the biological minimum required for seed germination (Otorepec, 1980). It grows well on poor soils, but does not

[1]Jasmina Balijagić (corresponding author: jas.be@t-com.me), Miodrag Jovančević, Irfan Mujanović, University of Montenegro, Biotechnical Faculty, MONTENEGRO, Jovan Crnobarac, University of Novi Sad, Faculty of Agriculture, Republic of SERBIA

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

provide high-quality yields as in deep soils, with average amount of nutrients. The plants grown under optimal conditions have a higher tolerance to stress factors (Kastori et al., 2013). A significant effect of irrigation on marigold yield has been noticed by Pirzada and Shokran (2012). Adamović (2011) raised the possibility of marigold double cropping, while Popovic et al. (2017) indicated the positive effect of marigold cultivation on poor and neglected soils, with extensive cultivation practices and minimal investments. Pot marigold blooms from June until the first strong frosts. The blossoms are orange or yellow-orange (Stepanovic et al., 2011). The pot marigold flowers yield depends on locality, variety selection and stand density (Hojden et al., 1990; Biesiada et al., 2006; Crnobarac et al., 2008; Parađiković et al. 2013). Since it, leaves the land early, the pot marigold is a good pre-crop to other cultures in crop rotation (Kišgeci, 2008). For the pot marigold growing, it is necessary to use the seed that gives abundant flower yield, quality flower heads (*Calendulae anthodium*) or tongue-shaped ray or ligulate flowers (*Calendulae flos*) rich in active substances, as well as varieties resistant to diseases and pests (Baciu et al., 2010). The pot marigold flower is used in medicine, including the traditional medicine, and in cosmetic industry (Pirzada and Shokran, 2012; Torbaghan, 2012). Even dried flowers of pot marigold should maintain their lovely orange colour. Moreover, it should have a discreet, pleasant scent. The drug must be free of any organic or inorganic additives (Jevđović et al., 2011). It belongs to the oldest medicinal plants that have been used from the ancient times (Król, 2011), but pot marigold is also used for decorating green spaces (parks) and in various floral arrangements (Selaru, 2007).

Pot marigolds are planted out in early spring, usually by mid-April. Crnobarac et. al. (2011) carried out the sowing in the area of Novi Sad on April 13, whereas in the research conducted by Parađiković et al. (2013), sowing was carried out in Brod-Posavina County on 22 May. Sowing date forecast achieves best results when there is a possibility of comparative measurements of meteorological parameters on the production parcel (Otorepec, 1980).

In investigating the influence of variety and plant density on fresh flower yield and dynamics of yield formation per harvest Crnobarac et. al. (2008) reported that, on average for all varieties, the highest yield was achieved at 50 cm row spacing, and that why we decided to apply the same row spacing. Sowing was done manually at 4.0, 5.0, 6.7 and 10 cm distances in the row, at a depth of about 3 cm, with two seeds per hill. In 3- 5 leaves phase, plants were thinned to final plant distance in the row (Jaćimović et al., 2007).

There is a difference in yields of different varieties due to genetic differences between varieties, the influence of the environmental factors as well as the interaction between the genotype and environmental factors. Planting density is one of the important factor affecting yield components. Therefore, the aim of this paper is to show the influence of variety and distance in row on pot marigold yield in agro-ecological conditions of Bijelo Polje (Montenegro).

MATERIAL AND METHODS

The field experiment was conducted in 2010 at experimental field in Lješnica. Three varieties were investigated: Plamen Plus (from the Czech Republic), Bački Petrovac and Orange King (material originating from Serbia)**.** The crop was sown on 8 May 2010 at 50 cm spacing between the rows. Due to climatic characteristics at the experimental site, sowing was carried out on 8 May. During the growing season, standard crop maintenance measures were carried out (hoeing, weeding). The flowers were plucked by hand in 7 successive harvest time, beginning from 17 July.

The variance analysis was used to process the obtained results, in line with the plan of setting up the experiment, which was organized as a three factor split-split design. The significance of differences between treatments was determined by using the LSD test at the significance threshold of 1% and 5%.

The experiment was established on weak acid and poor lime soil, very rich in humus with low content of available phosphorus and medium content of available potassium.

The experiment locality is on 650 m above sea level, with moderate continental type of climate. Unlike the ten-year average, in the year of setting up the experiment, total monthly precipitation in the vegetation period was not uniformly distributed, so that in August it was only 16 mm. In experimental year the highest daily mean and maximum air temperature occur in the seventh month (Table 1). The same as in the long-term average.

Table 1. The monthly sum of rainfall (mm) and average monthly air temperature (°C) in 2010 at Bijelo Polje

Months	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**
Total monthly rainfall	101.3	80.0	69.7	79.8	79.6	56.2	85.1	16.0	80.0	68.4	131.1	147.3
Maximum monthly temperature	18.0	14.8	22.8	25.6	27.9	34.1	33.9	36.0	30.6	23.2	22.8	19.5
Mean monthly air temp.	-2.4	-2.2	1.4	5.8	10.5	14.4	15.6	15.0	10.8	7.2	2.2	-0.6

*Source: Hydrometeorological Institute of Montenegro

RESULTS AND DISCUSSION

The analysis of the fresh flowers yields in each harvest indicate that the harvest time did not have statistically significant impact on yield, and yields were stable in all 7 harvests. The lowest yield in our experiment was accomplished in the fourth harvest due to increased air temperature and reduced precipitation (Tab. 1) which is in accordance with statements by Kastori et al. (2013) that, among natural factors, water stress and extreme air temperatures most often affect the plant growth. The yield of flowers in average and in each harvest was the highest on variety Plamen Plus (818 kg ha^{-1}), compared to other two varieties that did not differ significantly.

This is observed as regularity in almost all the harvests. The highest yield in average for all varieties and harvests amounted to 654 kg ha^{-1} in a single harvest and it was obtained at the minimum distance in row of 4 cm. It should be emphasized that from the second to the fifth harvest, the effect of plant distance in the row was different, indicating a stronger interaction. Variety Plamen plus in average obtained statistically highest yield in each harvest at 4 cm distance in row (1000 kg ha^{-1}), and variety Orange king at 6.7 cm distance in row, whereas distance in row did not have a statistically significant effect on the variety Bački Petrovac.

Figure 1. The application of agro-technical measures during the experiment (Photo: Jasmina Balijagić)

Table 2. Effect of harvest time, variety and distance in row on pot marigold fresh flower yield (kg/ha)

Harvest (**H**)	Variety (**V**)	Distance in row cm (**D**)				Average **HV**	*Average* ***H***
		4	5	6.7	10		
1	PLAMEN PLUS	1221	1061	619	1216	**1029**	***643***
	BAČKI PETROVAC	898	245	550	713	**602**	
	ORANG KING	349	117	457	267	**298**	
	Average **HD**	**823**	**474**	**542**	**732**		
2	PLAMEN PLUS	909	752	740	1218	**905**	***629***
	BAČKI PETROVAC	544	236	522	482	**446**	
	ORANG KING	576	172	869	528	**536**	
	Average **HD**	**676**	**386**	**710**	**743**		
3	PLAMEN PLUS	1027	532	694	784	**759**	***590***
	BAČKI PETROVAC	469	820	655	527	**618**	
	ORANG KING	358	271	577	368	**394**	
	Average **HD**	**618**	**541**	**642**	**560**		
4	PLAMEN PLUS	286	732	832	803	**663**	***510***
	BAČKI PETROVAC	274	677	362	657	**493**	
	ORANG KING	206	273	721	291	**373**	
	Average **HD**	**255**	**561**	**638**	**584**		
5	PLAMEN PLUS	904	862	500	678	**736**	***549***
	BAČKI PETROVAC	510	912	299	434	**539**	
	ORANG KING	296	718	168	313	**374**	
	Average **HD**	**570**	**831**	**322**	**475**		
6	PLAMEN PLUS	1213	604	475	790	**771**	***561***
	BAČKI PETROVAC	330	123	316	444	**303**	
	ORANG KING	639	658	557	584	**610**	
	Average **HD**	**727**	**462**	**449**	**606**		
7	PLAMEN PLUS	1440	515	664	825	**861**	***643***
	BAČKI PETROVAC	494	224	216	730	**416**	
	ORANG KING	788	435	824	557	**651**	
	Average **HD**	**907**	**391**	**568**	**704**	*Average* ***V***	
Average **VD**	PLAMEN PLUS	1000	722	646	902	***818***	**<u>589</u>**
	BAČKI PETROVAC	503	462	417	570	***488***	
	ORANG KING	459	378	596	415	***462***	
	Average ***D***	***654***	***521***	***553***	***629***		

LSD	Harvest	Variety	Distance	Harvest* Variety	Harvest* Distance	Variety* Distance	Harvest* Variety* Distance
1%	237	148	130	384	370	242	638
5%	173	111	99	289	280	183	484
F probability	0.591	<.001	0.027	0.082	<.001	0.002	

Table 3. Effect of variety and distance in row on pot marigold fresh flower total yield of all harvests

Variety (**V**)	Distance in row (**D**)				Average **V**
	4	5	6.7	10	
PLAMEN PLUS	7000	5057	4524	6314	**5724**
BAČKI PETROVAC	3519	3237	2920	3987	**3415**
ORANG KING	3212	2644	4173	2908	**3234**
Average **D**	**4577**	**3646**	**3872**	**4403**	

LSD	Variety	Distance	Variety* Distance
1%	820	886	1444
5%	541	656	1075
F probability	<.001	0.023	0.023

The study of the effects of the variety and plant density on a total fresh flowers yield of all harvests (Table 3) has shown that the variety has a statistically significant effect on yield. Variety Plamen plus had a significantly higher yield (5724 kg ha^{-1}), in relation to other two varieties which did not differ significantly, although the variety "Orange king" obtained the lowest yield (3243 kg ha^{-1}). Crnobarac et.al. (2008) also found that fresh flowers yield in average and on each row spacing was the highest on variety Plamen Plus. Although there were significant differences in yield affected by distance in row, there is no general regularity of its effect, due to the high interaction with varieties. Namely, the variety Plamen plus gave significantly highest yield at 4 cm distance in row, variety Orang king at 6.7 cm distance in row, while distance in row did not significantly affect the variety Bački Petrovac, although the yield was highest at 10 cm distance. A significant effect of plant density on yield was also determined by Parađiković et al. (2013).

CONCLUSIONS

According to field experiment data of influence of variety and distance in row on pot marigold yield in agro-ecological conditions of Bijelo Polje it could be made following conclusions.

The municipality of Bijelo Polje represents area of hills and valleys along the rivers and this environment enable plantation and economically feasible yields of pot marigold flowers.

The highest yield in average was recorded by variety Plamen plus, at all applied distances in row. Variety Plamen Plus obtained the highest yield at 4 cm distance in row.

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Pavel SOLONECHNYI, Mikhail KOZACHENKO, Nataliya VASKO, Vladimir GUDZENKO, Vitaliy ISHENKO, Galina KOZELETS, Nadezhda USOVA, Yuriy LOGVINENKO, Aleksandr VINYUKOV[1]

AMMI AND GGE BIPLOT ANALYSIS OF YIELD PERFORMANCE OF SPRING BARLEY (*Hordeum vulgare L.*) VARIETIES IN MULTI ENVIRONMENT TRIALS

SUMMARY

We evaluated the phenotypic stability and adaptability of spring barley varieties using the additive main effect and multiplicative interaction (AMMI) and genotype main effect (G) plus genotype-environment (GE) interaction (G+GE) biplot models. The research was conducted in five locations by randomized complete block design with three replications in each location in 2015-2016. The grain yields of all nine barley genotypes were significantly affected by environment, which accounted for 90.5% of the total variation, whereas genotype and genotype-environment interaction accounted for 4.0% and 5.5%, respectively. The AMMI and GGE biplot models reflected most of the variation caused by genotype and genotype-environment interaction effects in the first two principal components – 78.8% and 79.3%, respectively. The AMMI stability value (ASV) revealed that varieties Khors (G4) and Poduv (G5) were stable. GGE-biplot "which-won-where" showed that 9 environments used for the study belonged to 3 mega-environments with wining varieties Vzirets (G1), Inkliuziv (G6) and Dokaz (G9). According to the ideal genotype biplot, varieties Dokaz (G9), Inkliuziv (G6) and Khors (G4) were the best genotypes demonstrating high average yields and high stability of performance across the test locations. The results finally indicated that AMMI and GGE biplot were informative methods to explore stability and adaptation patterns of genotypes in practical plant breeding and in subsequent variety recommendations.

Keywords: Spring barley, GGE biplot, AMMI analysis, yield

INTRODUCTION

Barley breeding is a time consuming process, which in many cases lasts over a decade. Selection of superior genotypes is one of the most important goal

[1]Pavel Solonechnyi (corresponding author: pashabarley86@gmail.com), Mikhail Kozachenko, Nataliya Vasko, Plant Production Institute nd. a V. Ya. Yuriev NAAS, Kharkov, UKRAINE; Vladimir Gudzenko, The V.M. Remeslo Myronivka Institute of Weat NAAS, Mironovka, UKRAINE; Vitaliy Ishenko, Galina Kozelets, Kirovograd State Agricultural Experimental Station of NAAS, Kropivnitskiy, UKRAINE; Nadezhda Usova, Institute of oilseeds crops of NAAS, Zaporozhzhye, UKRAINE; Yuriy Logvinenko, Aleksandr Vinyukov, Donetsk State Agricultural Experimental Station of NAAS, Krasnoarmeysk, UKRAINE

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in barley breeding. In order to evaluate the performance of these genotypes, plant breeders conduct field trials in different locations. These tests enable collecting data on genotype stability and adaptability (Mirosavljević, 2014).

When genotypes are tested for performance in several environments, the rankings usually differ as differences in environment may produce different effect on genotypes. Such inconsistent phenotypic performance of genotypes across environments is called genotype-environment interaction (Asfaw et al., 2009). Genotype-environment interaction (GEI) is differential phenotypic performance of genetically uniform genotypes across test environments. It occurs because different genotypes have different genetic potentials to adjust them selves to variable environments and causes one genotype to not win every where and always (Zeleke and Berhanu, 2016).

In this context, Multi Environment Trials (METs) are important for studying yield stability, adaptation and as well for prediction of yield performance of genotypes across environments (Solonechnyi at al., 2015). Typically, environment causes most of the total yield variations, while genotype and genotype-environment interaction (GEI) are usually less effective (Yan and Kang, 2003; Dehghani et al., 2010). A large GEI variation usually hinders the accuracy of yield estimation and reduces correlation between genotypic and phenotypic values. GEI is a universal phenomenon when different genotypes are tested in a number of environments, and is an important issue for plant breeders and agronomists to predict cultivar behavior in different locations across different years prior to any recommendation concerning varieties (Kang, 1998; Annicchiarico, 2002; Karimizadeh et al., 2012; Yan et al., 2007; Mortazavian, 2014).

Different methods are presented for statistical analysis of MET data, including parametric and non-parametric, to estimate the nature of genotype interactions with the environment and their control, but a method that would be approved by everyone has not still been introduced (Kaya et al., 2006).

Two frequently used statistical analyses are the additive main effects and multiplicative interaction (AMMI) model and the genotype main effects and genotype-environment interaction effects (GGE) model (Gauch, 2006). These two statistical analyses (AMMI and GGE) have broader relevance for agricultural researchers because they pertain to any two-way data matrices, and such data emerge from many kinds of experiments.

The AMMI model is a hybrid model involving both additive and multiplicative components of two way data structure which enables a breeder to precisely predict genotypic potentiality and environmental influences on it. AMMI uses ordinary ANOVA to analyze the main effects (additive part) and principal component analysis (PCA) to analyze the non additive residual left after ANOVA (Gauch, 1988; Zobel at al., 1988). Concerning the use of AMMI in METs data analysis, which partitions the GEI matrix into individual genotypic and environmental scores, an example was provided by Zobel et al. (1988).

Purchase et al. (2000) developed a quantitative stability value to rank genotypes through the AMMI model, namely the AMMI Stability Value (ASV).

Yan et al. (2000) proposed a modification of the conventional AMMI analysis called GGE (genotype and genotype-environment interaction) which is used for GEI analysis. The GGE analysis pools the genotype effect (G) with GE (multiplicative effect) and submits these effects to principal component analysis. According to Yan et al. (2000), this biplot is identified as a GGE biplot. GGE biplot has some graphical visualization function such as visualization of genotypes performance in a specific environment, visualization of relative adaptability of a genotype in a varying environment, visualization of comparison of two genotypes in different environment, visualization of identifying the best genotypes in every environment, visualization of an environmental group for a specific genotpe(s), visualization of genotype average performance and stability, and visualization of discrimination and representation of environment (Yan and Hunt, 2001). These aspects make GGE biplot a most comprehensive tool in quantitative genetics and plant breeding (Mousavi at al., 2016).

This objective of our study was to evaluate the adaptability and yield stability of spring barley varieties using GGE biplot and AMMI analysis to select varieties that have both high performance and phenotypic stability.

MATERIAL AND METHODS

Trials. Nine spring barley varieties bred at the Plant Production Institute nd. a V.Ya. Yuryev of NAAS were tested in nine environments, including five different locations, in 2015 and 2016. The locations were: Kharkov in the northeast of Ukraine; Mironovka in the north of Ukraine; Kropivnitskiy in the central part of Ukraine; Krasnoarmeysk and Zaporozhzhye in southeast of Ukraine (Table 1). The genotypes were grown by a randomized complete block design with three replications in each site.

Table 1. Code and name of 9 spring barley and 5 testing locations

Variety	Code	Location	Year	Code	Latitude	Longitude	Altitude (m)
Vzirets	G1	Kharkov	2015	E1	50°00' N	36°13' E	117
Agrariy	G2		2016	E2			
Alegro	G3	Mironovka	2015	E3	49°39' N	31°00' E	128
Khors	G4		2016	E4			
Poduv	G5	Zaporozhzhye	2015	E5	47°50' N	35°08' E	86
Inkliuziv	G6		2016	E6			
Modern	G7	Kropivnitskiy	2015	E7	48°30' N	32°16' E	113
Kozvan	G8		2016	E8			
Dokaz	G9	Krasnoarmeysk	2016	E9	48°16' N	37°10' E	181

ANOVA. Combined analysis of variance was performed for all the environments and included five locations and two years (data of Krasnoarmeysk were for one year). The treatment sum of squares was partitioned into its three

components: genotype (G), environment (E) and genotype-environment interaction (GEI).

GGE biplot analysis. The GGE biplot methodology, which is composed of 2 concepts, the biplot concept (Gabriel, 1971) and the GGE concept (Yan et al., 2000), was used to visually analyze the multi-environment yield trials (METs) data. This methodology uses a biplot to show the factors (G and GE) that are important in genotype evaluation and that are also sources of variation in GEI analysis of METs data (Yan et al., 2000, 2001).

AMMI analisys. Stability (AMMI stability value (ASV) was calculated according to the formula, as described by Purchase et al. (2000).

The data on yields were mathematically processed using Genstat12 software.

RESULTS AND DISCUSSION

ANOVA and AMMI analysis. Combined analysis of variance showed that there were highly significant differences for environment, genotype and their interactions. Combined analysis of variance and AMMI analysis are shown in Table 2. In our study, spring barley grain yields were significantly affected by the environment, which accounted for 90.5% of the total (G + E + GE) variation, whereas genotype and genotype-environment interaction accounted for 4.0% and 5.5%, respectively. A large sum of squares for environments indicated that the environments were diverse, with large differences between environmental means causing variation in the grain yields.

Table 2. Combined and AMMI analyses of variance and contributions of the first four principal components to the grain yield of 9 spring barley genotypes in 9 environments

Source	DF	SS	MS	G+E+GE SS (%)	GE SS (%)
Genotypes (G)	8	19.6	2.450**	4.0	
Environments (E)	8	442.5	55.228**	90.5	
Interactions (GE)	64	27.4	0.428**	5.5	
IPCA1	15	13.1	0.873		47.8
IPCA2	13	8.5	0.652		31.0
IPCA3	11	2.3	0.206		8.4
IPCA4	9	1.7	0.193		6.2
Residuals	16	1.8	0.115		
Error	144	4.5	0.031		
Total	242	494.0	2.041		

DF = degree of freedom; SS = sum of squares; MS = mean square; IPCA = interaction principal component axis; **significant at 1 %.

In most multi-environment trials the environment accountes for over 80% of the total variation (Yan, 2002; Yan and Kang, 2003; Gauch at al., 2008).

Combined ANOVA determines whether GEI is a significant source of variation or not and estimates it, but does not provide insight into the patterns of genotypes or environments that give rise to the interaction (Samonte et al., 2005). Therefore, the combined data were also analyzed using the AMMI model that further partitions GEI into IPCA (Interaction Principal Components Axes) components. The results from AMMI analysis also show that the first and second principal component axis accounted for 78.8% of the interaction variability, enabling us to evaluate the stability of genotypes for these two components.

Table 3 shows the AMMI model IPCA1 and IPCA2 scores of the grain yield for 9 spring barley genotypes in 9 environments and the AMMI stability value (ASV) for each genotype. Purchase et al. (2000) developed a quantitative stability value to rank genotypes through the AMMI model, named the AMMI Stability Value (ASV). According to ASV ranking, varieties Khors (G4) and Poduv (G5) were the most stable genotypes, while genotype Vzirets (G1) was unstable.

Table 3. Mean grain yield (tha^{-1}), first and second principal component axis (IPCA) and AMMI stability values (ASV) for the grain yield of 9 spring barley genotypes in 9 environments

Code	Mean	IPCA1	IPCA2	ASV
G1	4.68	-1.217	0.067	2.284
G2	4.46	0.335	0.626	0.504
G3	4.60	0.330	-0.620	0.553
G4	4.95	0.143	0.020	0.031
G5	4.78	-0.022	0.028	0.002
G6	4.98	0.281	-0.173	0.152
G7	4.12	0.481	0.264	0.425
G8	4.33	-0.195	0.517	0.326
G9	4.99	-0.136	-0.728	0.558
Mean	4.65			
LSD_{05}	0.09			

GGE biplot analisys. The first two principal components of GGE biplot model obtained by singular value decomposition of the centered data of grain yield accounted for 79.3% of the total variability caused by G + GE effects. Out of these variations, PC1 and PC2 accounted 52.2% and 27.1% variability, respectively.

Visualization of the “which-won-where” pattern of MET data is important for studying possible existence of different mega-environments (ME) in a region (Gauch and Zobel, 1997; Yan et al., 2000, 2001). The polygon view of a GGE

biplot explicitly displays the "which-won-where" pattern and, hence, is a succinct summary of the GEI pattern of a MET data set (Figure 1). By connecting the genotype markers and the rays as depicted, the rays in Figure 1 are lines that are perpendicular to the sides of the polygon or their extensions. These 6 rays divide the biplot into 7 sectors, but environments fall into three of them, so the genotypes vertex in these sectors may indicate higher or the highest yield compared to other parts in all environments (Yan, 2002).

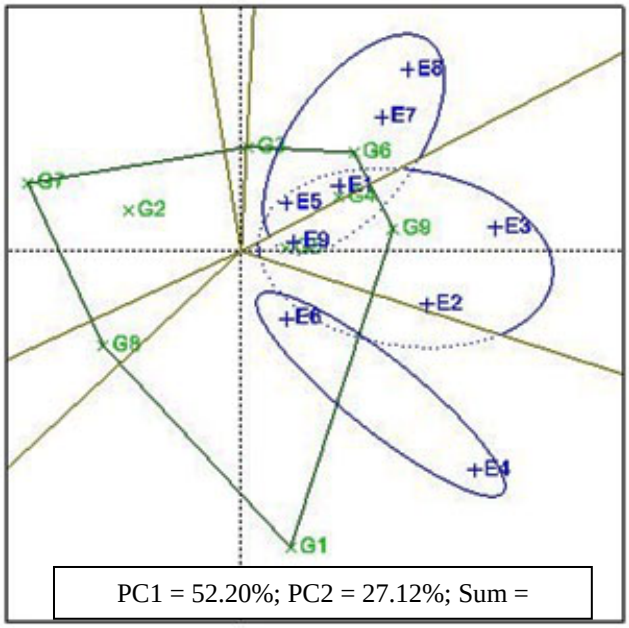

Figure 1. Polygon views of the GGE biplot based on symmetrical scaling for the «which-won where» pattern for genotypes and environments.

Another important feature of this biplot is that it indicates environmental groupings, which suggests a possible existence of different mega-environments. Thus, in our studies the first mega-environment consists of environments E8, E7, E5 and E1 with variety Inkliuziv (G6) being the winner. The environments E3, E2 and E9 makes up the second mega-environment, where variety Dokaz (G9) is winner. The last mega-environment consists of environments E4 and E6, where variety Vzirets (G1) has the highest yield capacity.

The yield stability of genotypes was evaluated by the average environment coordination (AEC) method (Yan, 2001; Yan and Hunt, 2001; Yan, 2002). In this method, the average principal components will be used in all environments, as depicted in Figure 2. A line is then drawn through this average environment and the biplot origin; this line is called the average environment axis and serves as the abscissa of the AEC. Unlike the AEC abscissa, this has one direction, with the arrow pointing to a greater genotype main effect; the AEC ordinate in either direction away from the biplot origin indicates a greater GEI effect and reduced

stability. The AEC ordinate separates genotypes with below-average means from those with above-average means. Varieties Dokaz (G9), Khors (G4) and Inkliuziv (G6) had the highest mean yields, varieties Modern (G7) and Kozvan (G8) – the lowest mean yields. The yield of variety Vzirets (G1) was the most variable, while varieties Khors (G4), Poduv (G5) and Dokaz (G9) were noticeable for their high stability.

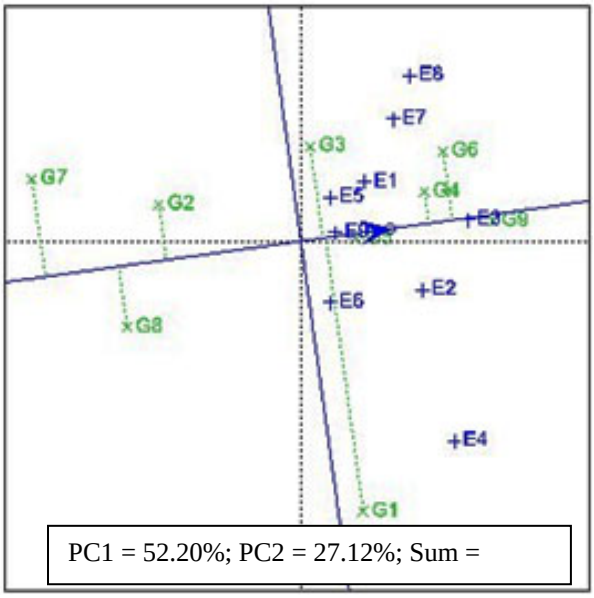

Figure 2. Average environment coordination (AEC) views of the GGE biplot based on environment-focused scaling for the mean performance and stability of genotypes

Stability in itself, however, is not the only parameter of genotype evaluation, because the most stable genotypes do not necessarily have high performance (Mohammadi at al., 2007; Mohammadi and Amri, 2008). An ideal genotype is one that has both high mean yield performance and high stability. The centre of concentric circles (Figure 3) represents the position of an ideal genotype, which is defined by a projection onto the mean-environment axis that equals the longest vector of the genotypes that had above-average mean yields and by a zero projection onto the perpendicular line (zero variability across all environments). The closer a genotype to the ideal one is the more valuable it is. Although such an ideal genotype may not exist in reality, it can be used as a reference for genotype evaluation (Yan and Tinker, 2006). Thus, varieties G7, G2, and G8 which had fell below the AEC ordinate, showed below average seed yield performance, whereas varieties G1, G3, G8, G4, G6 and G9, which fell above the AEC ordinate, performed above average. Varieties Dokaz (G9), Inkliuziv (G6) and Khors (G4) which had performed above average and had relatively shortest projection vectors from AEC line, were both high yielding and widely adapted.

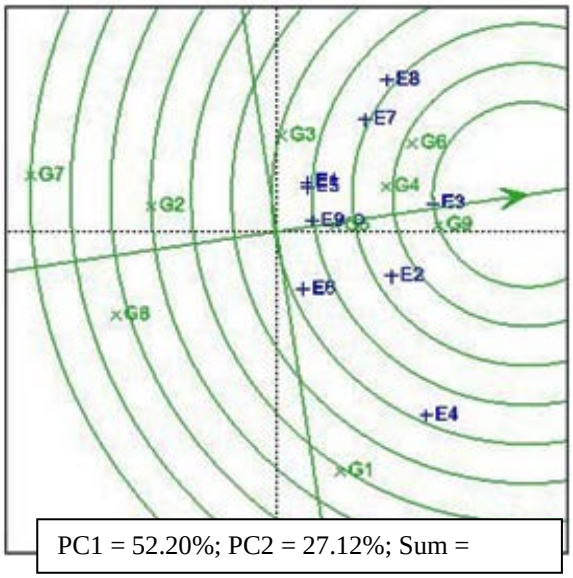

Figure 3. GGE biplot based on genotype-focused scaling for comparison the genotypes with the ideal genotype.

GEI reflects differences in adaptation and can be exploited by selecting for specific adaptation if the trend in specific adaptability of genotypes is repeatable over years (Annicchiarico, 2002; Yan et al., 2007). However, in this study, the specific adaptability trend was not repeated over years as different environments were grouped differently in the two years. Therefore, GEI couldn't be exploited and should be minimized by selecting for broad adaptation. Thus, broadly adapted varieties, G9, G6, G4 and G7, were recommended for verification and release.

Discriminating ability, representativeness and relationships of the test environments. According to Yan et al. (2007), due to the discriminative ability and representativeness of GGE view, the biplot was an effective tool for environment evaluation, which was not possible with the AMMI model. In environment focusing scaled vector view of GGE biplot, the cosine of the angles between environment vectors show relationships between test environments: with acute angles indicating strong positive correlation, obtuse angles – strong negative correlation or cross over GEI of genotypes, and right angle showing no correlation (Yan and Tinker, 2006). Hence, highly correlated environments E9 with E3, E6 with E4, E5 with E7 and E8 (Figure 4).

Discriminating ability and representativeness of the testing environments are an important measure in the GGE biplot. An environment is more desirable if it is located closer to the ideal environment. Thus, using the ideal environment as the centre, concentric circles were drawn to help visualize the distance between each environment and the ideal environment (Yan et al., 2000; Yan and Rajcan, 2002).

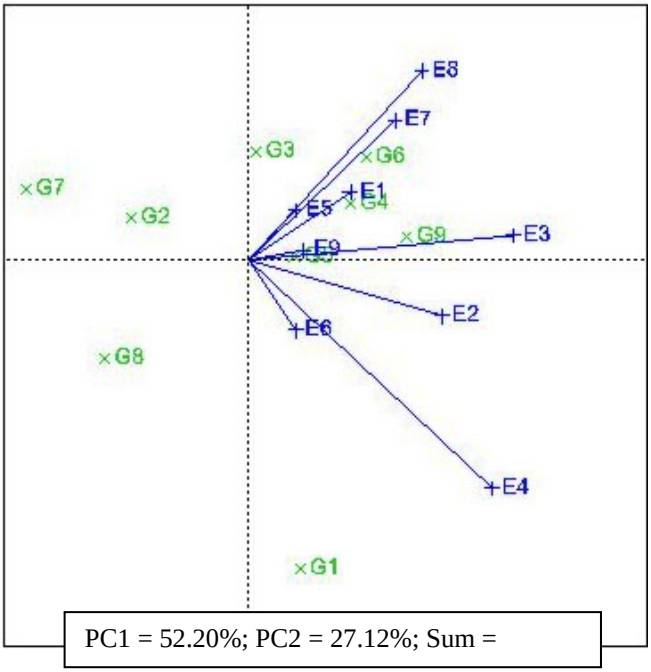

Figure 4. GGE biplot showing relationships among the test environments

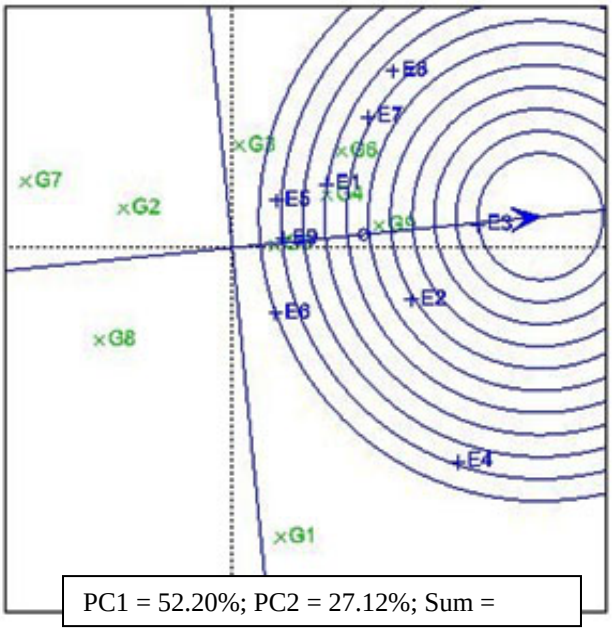

Figure 5. Ranking of environments based on discriminating ability and representativeness

Figure 5 shows that environment E3 was the ideal test environment in terms of being the most representative of the overall environment and was chosen to select superior genotypes. Environments E6, E5 and E9, closer to the biplot origin, are characterized by similar performance of all genotypes; hence they provide little or no information about the genotypic differences, therefore, similar test environments should not be considered as test environment for yield trials. E8 and E4 have long vectors and large angles with the abscissa, hence, should not be used for selecting superior genotypes, but useful for culling unsuitable genotypes.

CONCLUSIONS

The result showed that the magnitude of the environmental effect was by far higher than the genotype effect and genotype-environment interaction effect. Spring barley varieties evaluated in this study had highly significant genetic differences in the grain yield performance across the environments.

Varieties Dokaz, Inkliuziv, Khors and Poduv are characterised by the highest mean yield and high stability and are expected to have the greatest commercial success. In terms of the regional distribution, it should be emphasized that it has a predictive character and requires continued multi-year testing.

The results finally indicated that AMMI and GGE biplot were informative methods to explore stability and adaptation of genotypes in practical plant breeding and in subsequent variety recommendations.

ABREVIATION

AMMI – additive main effects and multiplicative interaction; GGE – genotype main effects and genotype-environment interaction effects; AEC – average environment coordinate; PCA – principal components analysis; SVD – singular value decomposition; GEI – genotype-environment interaction.

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Livija MAKSIMOVIĆ, Vera POPOVIĆ, Petar STEVANOVIĆ [1]

WATER AND IRRIGATION REQUIREMENTS OF FIELD CROPS GROWN IN CENTRAL VOJVODINA, SERBIA

ABSTRACT

Amount and distribution of precipitation and mean daily air temperatures were analyzed in the period of 30 years (1987-2016) in Bački Petrovac, situated in the vicinity of Novi Sad, central Vojvodina. Increase in mean daily air temperatures was recorded during the whole vegetation period, 5.7% on average compared to the previous period (1948-1990). According to the water balance used as the basis for drought estimation, regular and increased deficit in plant-available water was observed mainly in July and August, despite the recorded sum of precipitation equal to or slightly higher than the previously obtained average. High oscillations of field crop yields, mainly due to different weather conditions in specific growing seasons, had been observed across the growing years. Average yields recorded in the period 2006-2014 for most significant field crops - maize, sugar beet, sunflower and soybean, were higher compared to the previous period by 2.16%, 17.81%, 19.39% and 38.71%, respectively. Average yields in Republic of Serbia were as follows: maize 5.19 t ha^{-1}, sugar beet 46.42t ha^{-1}, sunflower 2.34t ha^{-1}, and soybean 2.58t ha^{-1}. The yields varied between 12.09% and 24.49%. Analyzed meteorological data indicate the need for irrigation, which would maintain and improve soil fertility, regulate soil water and nutrient regime, and thus provide the basis for a more successful plant production.

Keywords: air temperatures, drought, plant water requirements, precipitation, water deficit, yield.

INTRODUCTION

Climate, soil and yield are inextricably intertwined in natural environments and should therefore be adjusted in order to produce profitable, high-quality food.

The success of plant production is affected by many factors which vary from one to the other production season. Some of them can be altered using genetics, breeding, selection, cultivar classification (Pavićević, 1976; Dragović, 1997; Popović et al., 2015a, 2015b, 2016a, 2016b; Živanović et al., 2017) or by applying various cultivation practices in production technology. Soil quality can

[1]Livija Maksimović, Vera M. Popović, (corresponding authors: livija.maksimovic@nsseme.com; vera.popovic@nsseme.com), Institute of Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad, SERBIA; Petar Stevanović, Inspection Affairs Administration of Republika Srpska, Banja Luka, BOSNIA AND HERZEGOVINA;

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

also be improved by applying soil management practices, such as fertilization, organic matter introduction, soil tillage, a melioration, drainage, irrigation, and so on. Weather conditions, which is the most important factor of successful plant production, can be least altered.

It is necessary to monitor and analyze all the parameters in the process of plant production so as to observe the negative trends which could adversely affect the stability and quality of production. In such case, cultivation practices should be applied in order to halt or slow the negative processes and thereby maintain or improve plant production.

Agro-meteorological research are mainly related to: studies and estimations of changes in agro-climatic potential based on climate change and oscillations; studies of meteorological occurrences causing significant damage in agriculture in order to decrease their negative effects; improvement of operative agro-meteorology, especially the introduction of crop/time and time/plant disease models into practice in order to increase the quality of agro-meteorological analyses and forecasts; trial performance at lysimeter stations, etc (http://www.hidmet.gov.rs/latin/meteorologija/agro.php).

Great damage caused by drought in different industrial sectors (agriculture, water treatment and energy industry, etc) causes the need to take suitable precautions in order to reduce drought risks and related negative effects. Each plan to combat drought has three main components: monitoring and early announcement, estimating risks and reducing the negative effects. Permanent monitoring of all relevant parameters (temperatures, precipitation, insulation, wind, evapotranspiration, soil moisture, groundwater level etc.) is necessary, as it enables timely overview of the first signs of drought occurrence (Spasov, 2003).

The study analyzed climate parameters which significantly affect soil and plant water and nutrient regime, primarily mean daily air temperatures (largely influencing potential evapotranspiration and plant water requirements) and precipitation sum as the main plant water and nutrient source. Serious disturbance in hydrological balance negatively affects soil production capabilities. Mutual effects and relationships between climate parameters, plants and soil, make irrigation necessary as it is a cultivation practice which most successfully regulates plant-available water deficit in plant production.

MATERIAL AND METHODS

The amount and distribution of precipitation and mean daily air temperatures in the 30 year period (1987-2016) were analyzed in Bački Petrovac, near Novi Sad in central Vojvodina (φN 45° 20′, λE 19° 40′, 82 m a.s.l.). Potential evapotranspiration (ETP) i.e. plant water requirements were determined according to the results. Water requirements of the main field crops were calculated according to the biological method by Dragović (Maksimovic and Dragovic, 2002), using changed crop coefficients (i_c) depending on the development stage of the cultivated crop and temperatures (t °C) in a given month, by the equation:

$$ETP = \sum t\ °C \cdot i_c$$

Water balance, the relationship between water gain and water loss, was used in order to analyze the outbreak and intensity of drought. Differences between precipitation (P) and potential evapotranspiration (ETP) were calculated so as to obtain the level of climatic precipitation deficit required for undisturbed plant water supply (P-ETP). Deficit of available water in soil, needed to satisfy water requirements of the most frequently grown crops, also expresses irrigation needs which is the most efficient hydro-ameliorative measure for combat against drought.

RESULTS AND DISCUSSION

Mean daily air temperatures affecting potential evapotranspiration and plant water requirements were analysed for the region of central Vojvodina, as well as precipitation as the main plant water source containing dissolved nutrients. Sum and distribution of precipitation across short periods, years and growing seasons, is of particular importance, especially during the critical phases of plant development.

Analysis of temperature conditions in the air at ground level and moisture conditions (relationships between the amount of precipitation, evapotranspiration, and soil moisture) results in the obtained values of agro-climatic parameters and indexes determining climate conditions in plant production. Values of parameters used for agroclimatic resource estimation are obtained from processing multiannual data sets usually for a vegetation period and its parts. Mean air temperatures of 18.6°C were observed in this area during the vegetation period according to the 30-year data (1987-2016) collected in Bački Petrovac (Tab. 1).

Tab. 1. Mean monthly air temperatures (°C) at weather station Bački Petrovac across the tested periods

Months	Average 1948-1990	1987-1996	1997-2006	2007-2016	Average 1987-2016
April	11.4	11.5	11.9	12.7	12.0
May	16.5	16.6	18.2	18.3	17.7
June	19.6	19.8	21.1	21.4	20.8
July	21.1	22.1	22.3	23.2	22.5
August	20.5	21.5	21.8	22.4	21.9
September	16.7	16.8	16.7	17.3	16.9
Vegetation period	**17.6**	**18.0**	**18.7**	**19.2**	**18.6**

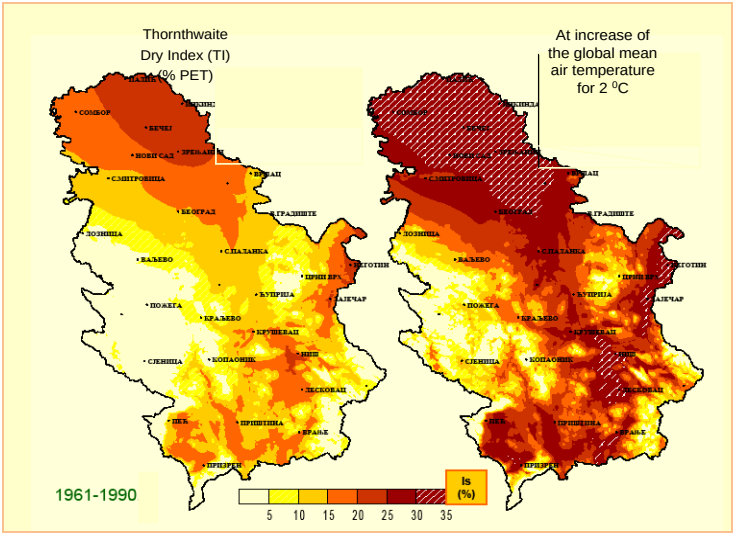
Compared to data from the previous period (1948-1990) obtained in the studies of numerous researchers (Hadžić et al., 1996), increase in mean daily air temperatures is observed in all the months, especially July and August (1.4°C) as well as throughout the whole vegetation period (1.0°C) (Tab. 1).

Evident increase in mean daily air temperatures has been observed in the last few decades, with the warmest period in 2007- 2016 when the recorded air temperature means were 19.2°C (Tab. 1).

Monthly temperature increase, as compared to the previous period 1948-1990, was: April-1.3; May-1.8; June-1.8; July- 2.1; August-1.9 and September 0.6°C. Experts of the Republic Hydrometeorological Institute of Serbia predicted the increase in Thornthwaite drought index (Is=TI) during moisture/drought monitoring in Serbia, due to global increase in mean daily air temperatures by 2°C (http://www.hidmet.gov.rs/podaci/agro/ciril/klipro_agrorhmzs.pdf).

The changes indicate significantly higher deficit of available water in soil (Graph. 1). Plant potential evapotranspiration is also increased by about 20%.

Studying the Thornthwaite precipitation-efficiency index, used to identify the characteristics of arid and humid climate, Selek et al. (2017) found that semi-arid zones in Turkey had significantly expanded (about 14% increase) between the two 30-year periods (1950-1980 and 1981-2010). By using the Thornthwaite precipitiation efficiency index, Li et al. (2016) recorded lower drought intensity in the last 53 years, which indicates certain benefits in the crisis of water use at the territory of Xinjiang in China. Kalaiarasi and Vipulanandan (2010) determined seasonal drought intensity and drought severity through Thornthwaite precipitation-efficiency index with a better correlation with annual precipitation sum than the suction pressure measured in the active zone.



Graph. 1. Thorntwaite dry index increase based on mean daily air temperatures increase by 2°C, 1987-2016

Thornthwait water balance is used to determine the level of precipitation required for evaporation and transpiration. According to Maksimović et al. (2017) potential evapotranspiration (ETP) for central Vojvodina is 744 mm,

while real evapotranspiration (ETR) is 562 mm. Water deficit for plant requirements is increased, especially in July and August, despite precipitation sum equal to or slightly higher than the sum in the vegetation period during the previous period of observation (346.5 mm in the previoud compared to 371.4 mm in the last 30 years). Water deficit of 181 mm should be added by irrigation, so as to improve soil water balance and plant nutrient regime. Moreover, it would preserve and improve fertility of arable land, provide better plant nutrient regime as well as stabilize and increase crop yields and their quality (Sikora et al., 2015).

Mean sums of potential evapotranspiration (mm) in Vojvodina according to Penman Monttheith for the period 1971-2000 are connected to temperature conditions (http://www.hidmet.gov.rs/latin/meteorologija/pros_pet.php) and they vary slightly compared to the Novi Sad data, so that the data collected in Bački Petrovac can be considered as the average for Vojvodina due to its close proximity to Novi Sad and less variation of the fundamental climatic condition.

Agronomic sources define drought as insufficient plant water supply from precipitation and other sources i.e. potential evapotranspiration (ETP). Drought can occur as soil, air, and physiological drought, which are interconnected and mutually dependent. Soil drought is highly significant for agriculture as it causes temporary or permanent water deficit, visibly limiting and reducing plant growth and development, decreasing organic matter production and crop yield (Dragović, 1997). Special attention is therefore given to soil water balance in the study, used for determination of water deficit by comparison of plant water requirements and precipitation-derived water gain. The deficit can be mitigated to some extent by the multidisciplinary approach to this issue, although irrigation is the most reliable method of drought combat.

Precipitation sums in the vegetation period are very variable across growing seasons, with much more pronounced fluctuations in the last six years compared to the previous study period. The percentage of precipitation recorded in relation to the multiannual average varies and ranges from 7% in August 2012 to 268% in May 2014 (Graph. 2).

Precipitation means during the vegetation period are higher by an average of 24.2 mm when comparing the periods 1987-2016 and 1948-1990 in central Vojvodina (Tab. 2, Graph. 3).

In the analysis of precipitation periods in 1971-2000, Spasov (2003) observed that precipitation decrease is less expressed in the growing seasons, while decrease in precipitation was not observed on an annual basis. This is a more favourable situation for plant production (Graph. 3) compared to forecasts provided by different hydro-meteorological models, according to which increased air temperatures and decreased precipitation is to be expected in this area, especially during vegetation periods (IPCC, 2007; Lalić et al., 2011).

The main crop water requirements are calculated using the biological method elaborated by Maksimović and Dragović (2002), through changing the plant coefficient (i_c) depending on the development stage of the cultivated crop and temperature conditions (t°C) in a given month, by the equation

$$ETP = \Sigma t°C \cdot i_c.$$

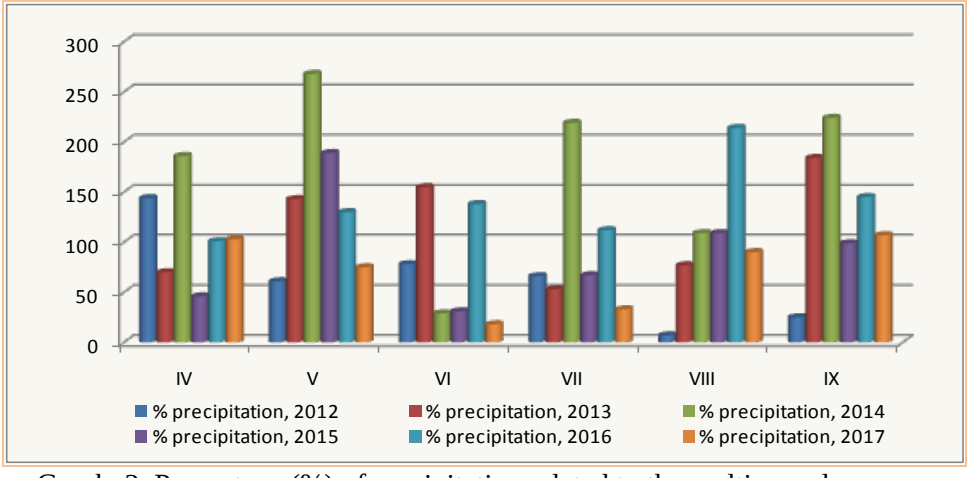
Research by Cipris and Evtushenko (1980), cit. Maksimović and Dragović, 2001) confirm that the changes in biological coefficients, as well as the amount of water during the growth period, is highly correlated with weather conditions. Calculations using the mean values are shown for the central Vojvodina across periods of research (Tab. 3), and in ten year intervals (Tab. 4). The month of April is excluded from calculation since it is the time when plants are sown, sprout or go through initial stages of development, when transpiration is very low.

The highest mean water requirements (ETP) are observed in sugar beet, followed by maize and sunflower, and the lowest in soybeans. Water deficit follows this trend and it is highest in sugar beet (with approximately 50% of sugar beet water requirements during the growing season). All major crops have had increased water requirements during the last thirty years (1987-2016) compared to the previous period of research (1948-1990).

The highest mean water requirements (ETP) are observed in sugar beet, followed by maize and sunflower, and the lowest in soybeans. Water deficit follows this trend and it is highest in sugar beet (with approximately 50% of sugar beet water requirements during the growing season). All major crops have had increased water requirements during the last thirty years (1987-2016) compared to the previous period of research (1948-1990).

Plant water requirements were constantly increasing during the observed decades (Tab. 4), reaching the amount of 600 mm for sugar beet, 526 mm for maize and sunflower, and 503 mm for soybeans. The values were determined in earlier periods with extremely dry and hot production seasons.

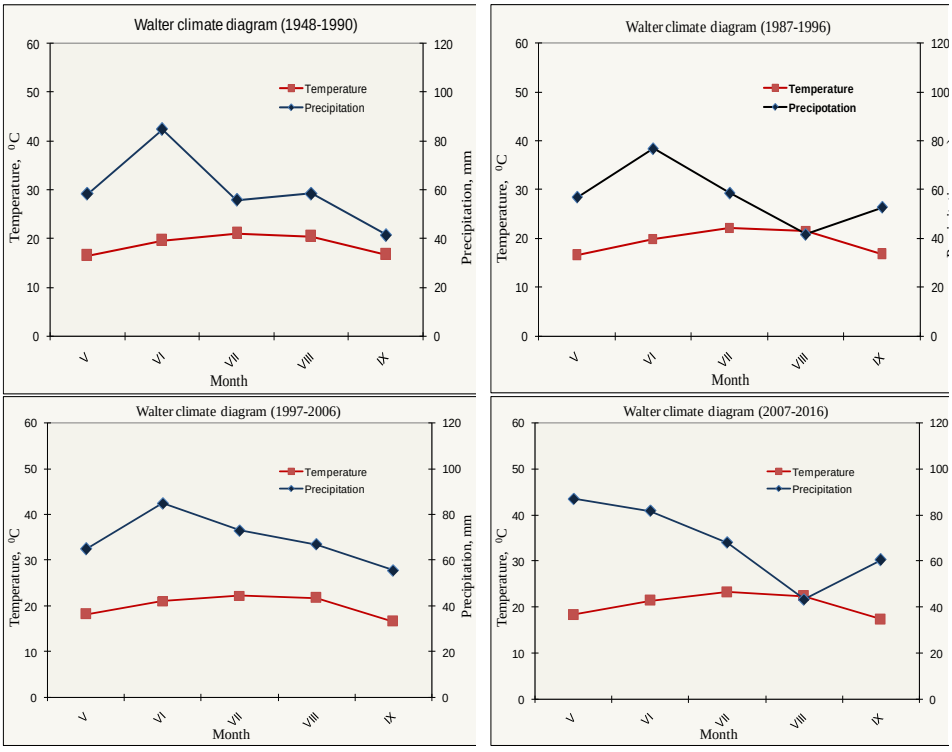
During the summer season (June, July and August), water requirements of most cultivated crops were approximately 100 – 150 mm per month (Tab. 3-4).



Graph. 2. Percentage (%) of precipitation related to the multiannual average (mm) at weather station Bački Petrovac, 2012-2017

Table 2. Total precipitation (mm), Bački Petrovac, Serbia

Months	Average 1948-1990	1987-1996	1997-2006	2007-2016	Average 1987-2016
April	47.4	44.8	53.7	41.1	46.5
May	58.4	56.8	64.9	87.0	69.6
June	84.9	76.7	84.9	81.8	81.1
July	55.9	58.6	73.0	68.0	66.5
August	58.5	41.7	67.0	43.4	50.7
September	41.5	52.6	55.7	60.4	56.2
Vegetation period	**346.5**	**331.2**	**399.2**	**381.7**	**370.7**



Graph. 3. Walter climate diagram accross study periods, 1948-1990; 1987-1996; 1997-2006; 2007-2016;

Summer deficit of water required for unhindered plant growth and development is very pronounced in central Vojvodina. During the 30 years of study (1987 – 2016) conducted in Bački Petrovac, drought was observed in July and August, (Maksimović et al., 2017) in 83% and 87% of the years, respectively (Tab. 5). Moderately wet to wet conditions were observed in July in 36.6% of the studied years as it is the month with the maximum amount of rain in this area.

Table 3. Field crop water requirements ETP (mm) and water deficit according to water balance in the region of Vojvodina (Bački Petrovac), Serbia

Month	Average		Sugarbeet		Maize and Sunflower		Soybean	
	Tempe-rature	Precipi-tation	ETP	Deficit	ETP	Deficit	ETP	Deficit
1948-1990								
May	16.5	58.4	76.7	18.3	71.6	13.2	56.3	-
June	19.6	84.9	117.6	32.7	94.1	9.2	100.0	15.1
July	21.1	55.9	137.4	81.5	117.7	61.8	117.7	61.8
August	20.5	58.5	127.1	68.6	114.4	55.9	108.0	49.5
September	16.7	41.5	60.1	18.6	60.1	18.6	55.1	13.6
Average	18.9	299.2	518.9	219.7	457.9	158.7	437.1	140.0
1987-2016								
May	17.7	69.6	82.3	12.7	76.8	7.2	60.4	-
June	20.8	81.1	124.8	43.7	99.8	18.7	106.1	25.0
July	22.5	66.5	146.5	80.0	125.6	59.1	125.6	59.1
August	21.9	50.7	135.8	85.1	122.2	71.5	115.4	64.7
September	16.9	56.2	60.5	4.3	60.8	4.6	55.8	-
Average	18.6	324.1	549.9	225.8	485.2	161.1	463.3	148.8

Table 4. Field crop water requirements ETP (mm) and water deficit according to water balance in the region of Vojvodina (Bački Petrovac), Serbia

Month	Average		Sugarbeet		Maize, Sunflower		Soybean	
	Tempe-rature	Precipi-tation	ETP	Deficit	ETP	Deficit	ETP	Deficit
1987-1996								
May	16.6	56.8	77.2	20.4	72.0	15.2	56.6	-
June	19.8	76.7	118.8	42.1	95.0	18.3	101.0	24.3
July	22.1	58.6	143.9	85.3	123.3	64.7	123.3	64.7
August	21.5	41.7	133.3	91.6	120.0	78.3	113.3	71.6
September	16.8	52.6	60.5	7.9	60.5	7.9	55.4	2.8
Average	19.4	286.4	533.7	247.3	470.8	184.4	449.6	163.4
1997-2006								
May	18.2	64.9	84.6	19.7	79.0	14.1	62.1	-
June	21.1	84.9	139.3	54.4	113.9	29.0	120.3	35.4
July	22.3	73.0	145.2	72.2	124.4	51.4	124.4	51.4
August	21.8	67.0	135.2	68.2	121.6	54.6	114.9	47.9
September	16.7	55.7	60.1	4.4	60.1	4.4	55.1	-
Average	20.0	345.5	564.4	218.9	499.0	153.5	476.8	134.7
2007-2016								
May	18.3	87.0	85.1	1.9	79.4	-	62.4	-
June	21.4	81.8	141.2	59.4	115.6	33.8	122.0	40.2
July	23.2	68.0	172.6	90.8	143.8	75.8	143.8	75.8
August	22.4	43.4	138.9	95.5	125.0	81.6	118.0	74.6
September	17.3	60.4	62.3	1.9	62.3	1.9	57.1	-
Average	20.5	340.6	600.1	259.5	526.1	193.1	503.3	190.6

Table 5. Percentage of dry years according to precipitation sum in July and August 1987-2016 in Vojvodina (Bački Petrovac), Serbia

Precipitation sum, mm	July		August		Category
	No.	%	No.	%	
0-25	5	16.7	8	26.7	Extremely dry
26-50	11	36.6	10	33.3	Very dry
51-75	5	16.7	4	13.3	Dry
76-100	4	13.3	4	13.3	Moderately dry
	25	83.3	26	86.6	Dry years - total
101-125	-	-	2	6.7	Moderately humid
>126	5	16.7	2	6.7	Humid
Total	30	100	30	100	

Drought effect on crop production. Variations in precipitation during growing seasons as well as low water balance in soil are the main reasons of low and unstable yields in Vojvodina Province observed in some years of study. Even in shorter periods, precipitation deficit causes water deficit in soil, poor water and nutrient regime of cultivated crops. Previously established lower precipitation decrease in the growing season in Vojvodina still has negative effects on agriculture, considering that most of the Vojvodina surface receives less rainfall compared to other regions in Serbia (Spasov, 2003).

Analyzing the yields of field crops in Vojvodina Province in the period 1965-2003, Bošnjak (2004) reported very low yields of the analyzed crops. The reductions exceeded 50% of the yields that can be obtained by irrigation (Table 6). Dragovic (1999) found that the average yields were much below genetic yield potentials of these crops, which were realized under 50%.

Average yields of all four tested crops in the Republic of Serbia during the period 2006-2014, were higher than in the previous period by 2.16% in maize, 17.81% in sugar beet, 19.39% in sunflower and 38.71% in soybean (Tab. 6).

Table 6. Average yields, extreme yields, and variation coefficient

Crops	Average yield (t ha^{-1})		Yield extremes (t ha^{-1})		CV (%)	
	1965-2003	2006-2014	1965-2003	2006-2014	1965-2003*	2006-2014**
Maize	5.08	5.19	2.26-7.11	3.25-7.51	21.57	24.49
Sugar beet	39.40	46.42	24.71-49.16	35.94-54.71	14.36	12.09
Soybean	1.86	2.58	0.92-7.25	1.73-3.54	27.55	21.14
Sunflower	1.96	2.34	1.39-2.63	1.90-2.90	16.64	14.40
Faostat.org. 2017; *Bošnjak, 2004; **Calculated of authors						

Average maize yields were 5.19 t ha^{-1}, varying from 3.25 to 7.51 t ha^{-1}; sugar beet yields were 46.42 t ha^{-1}, varying from 35.94 to 54.71 t ha^{-1}; sunflower yields were 2.34 t ha^{-1}, varying between 1.90-2.90 t ha^{-1}, while soybean yields were 2.58 t ha^{-1}, varying between 1.73-3.54 t ha^{-1} (Tab. 6). Sugar beet and sunflower yields exhibited the lowest variation (CV=12.09% and CV=14.40%,

respectively). The largest variation was recorded in maize (CV=24.49%) and soybean (CV=21.14%) (Tab. 6).

CONCLUSIONS

Amount and distribution of precipitation and mean daily air temperatures were analyzed in the period of 30 years (1987-2016) in Bački Petrovac, situated in the vicinity of Novi Sad, central Vojvodina.

Increase in mean monthly air temperatures by 5.7% was observed during the whole growing period, compared to the earlier period (1948-1990), with oscillations from 1.2% in September to 7.3% in May. As it is a thermal process which depends on the air temperature, potential evapotranspiration (ETP) i.e. water requirements of the main field crops exhibited an increase. According to the results of water balance, as the basis of drought estimation, there was a regular and increased deficit of water needed to satisfy plant water requirements, which was especially notable in July and August, regardless of precipitation sum equal to or slightly higher than precipitation sum recorded during the growing period.

Great oscillations of field crop yields in Vojvodina were observed in the study years, which mainly occurred due to variable weather conditions during the production season.

Compared to the previous study period, average yields of the four crops studied in the period 2006-2014 were higher by 16.2% in maize, 17.81% in sugar beet, 19.39% in sunflower, and 38.71% in soybeans. Average yield of maize, sugar beet, sunflower, and soybean was 19.5 t ha^{-1}, 46.42 t ha^{-1}, 2.34 t ha^{-1}, and 2.58 t ha^{-1}, respectively. Yields ranged from 12.09% to 24.49%.

Meteorological data recorded in central Vojvodina indicate the need for increased irrigation. Irrigation would preserve and improve fertility of agricultural soils and regulate soil water and nutrient regime, which would provide the conditions for a more successful plant production in a positive correlation with the yield of the grain per unit area.

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Mehdi ABDOLAHI, Farid SHEKARI*,
Jalal SABA and Esmaeil ZANGANI [1]

SEED PRIMING WITH SALICYLIC ACID ENHANCED GAS EXCHANGES PARAMETERS AND BIOLOGICAL YIELD OF WHEAT UNDER LATE SOWING DATE

SUMMARY

This experiment was done to evaluate the effect of two planting dates and salicylic acid (SA) on wheat photosynthesis. Wheat seeds, cv. Alvand, primed with SA (0, 400, 800, 1200, 1600, 2000 and 2400 µM) at two planting dates (recommended planting date, 23 October, and late planting date, 22 November). Gas exchange parameters were measured in three growth stages (tillering, heading and grain filling). The highest and lowest rate of photosynthesis (P_N), stomatal conductance (g_s) and transpiration rate (E) of plants were observed in heading and grain filling stages, respectively. Seed pretreatment with SA enhanced photosynthetic parameters and carboxylation efficiency (CE), but, intercellular CO_2 concentration and water use efficiency (WUE) reduced by application of SA. It seems that application of SA had more effects on g_s and E than P_N. Among growth stages, the highest value of WUE was found in tillering and lowest in heading stage. Priming with SA compensated late sowing effects on plants P_N. Chlorophyll content, chlorophyll a/b ratio and CCI values significantly increased in SA treated plants. Results show that priming with SA may reduce ameliorative effects of late sowing on wheat plant biomass production. Among SA concentrations, 1200 µM had highest value in both planting dates.

Keywords: Carboxylation efficiency, Chlorophyll content, Photosynthesis rate, Water use efficiency.

INTRODUCTION

Photosynthesis and related gas exchange parameters influenced by many internal and external factors. For example, it is reported leaf ontogeny, heterophylly and position (Hejnak et al., 2014), age (Wang et al., 2014), seasonal changes and conditions (Ribeiro et al., 2009), sink effect (Nebauer et al., 2011) have considerable effects on photosynthesis rate and its regulation. Level of leaf development and/or morphological and anatomical stage of plant may influence photosynthesis rate (Hejnak et al., 2014). Also, environmental history of leaves affect their photosynthetic development (Fitter and Hey, 2012).

[1]Mehdi Abdolahi, Farid Shekari*(corresponding author: shekari@znu.ac.ir), Jalal Saba and Esmaeil Zangani, Department of Agronomy, Faculty of Agriculture, University of Zanjan, IRAN
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Sowing date is a key factor on plants productivity potential and has deep effect on crop yield. It can by influence on plant tissues age change photosynthetic capacity of plants.

Salicylic acid (SA) is an endogenous growth regulator with phenolic structure, which participates in the regulation of different physiological and biochemical processes in plants (Raskin, 1992) and acts as an important signaling molecule (Nazar et al. 2011). SA, might play a role in g_s (Janda et al., 2014), photosynthesis (Fariduddin *et al*. 2003), and stomatal closure (Poor and Tari, 2012). Nazar et al. (2011) reported in mungbean SA increased photosynthesis under normal condition and alleviated salt effects on photosynthesis. These protective effects may be related with nitrogen and sulfur assimilation metabolism. Spraying of maize plants with SA led to increase photosynthesis, pigment content and growth rate (Khodary, 2004).

At many parts of Iran, because of unfavorable weather condition, rotation of wheat after late potato or maize cultivars and/or large planting areas, planting date may delay until late of autumn and these situations leads to weakly growth of seedlings or fail in establishment by cold stress damage. On the other hand, it is possible later growth and photosynthesis of plants after winter affected by pervious growth stages and growth history of plants. The aim of our experiment was to study the effects of two different sowing dates on gas exchange parameters and biological yield of wheat and possibility of ameliorative effects of seed priming by SA on these parameters.

MATERIAL AND METHODS

Seeds of wheat (*Triticum aestivum* L., cv Alvand) obtained from seed bank of faculty of Agriculture, University of Zanjan. Seed moisture content was 8.54% (based on dry weight). For all treatments, selected healthy seeds were used in same numbers.

Salicylic acid seed treatments

For seed priming, SA solutions prepared in six levels, including 400, 800, 1200, 1600, 2000 and 2400 µM. For each treatment SA powder (Merck, Darmstadt. Germany) weighted separately and solved in 5 cc ethanol and shaken well. Then solution added to 3 litter distilled water. The ratio of seed to solute was 1:5 (based on weight). Seeds submerged for 12 hours at 4 ºC. Then, seeds exposed to airflow and air-dried. Non primed seeds, were used as control treatment.

Planting and cultivation:

Seeds planted in two planting dates: 23 October (as conventional planting date in Zanjan province) and 22 November (as late planting date) of 2010 in research station of University of Zanjan (36˚ 40́ N; 48˚ 24́ E and 1610 m from sea level). In general, Iran has arid and semi-arid climate and the major precipitation occur from October to June (Table 1). The coldest month mainly occurs in January and cold weather and frost happen mid to end of November to March (Alijani, 2006). The day after planting plots irrigated and irrigation continued

until frizzing temperature was appeared. In spring from 4th of May irrigation again started normally each week. According to soil analysis 40 kg/ha phosphate in form of phosphate ammonia before planting and 80 kg/ha nitrogen in form of urea in two times (after seedlings emergence and before stem elongation) were added to field.

Table 1. Mean temperature of 10 years (from 1999-2009) and the experiment year (2009-10) of Zanjan region.

	2009-2010		1999-2009	
Months	Max	Min	Max	Min
October	27.2	-1.6	26.8	0.3
November	18.8	-7	18.4	-8
December	11.8	-8.4	12.4	-13.9
January	14.4	-15.4	10.5	-17.3
February	17.4	-12	12.8	-11.8
March	25.8	-4.2	20.3	-7.6
April	23	0.2	25	-2.6
May	29	1.2	29	2.1
June	35.6	9	34.4	5.9
July	40	10	36.1	10.1

Gas exchange

Gas exchange parameters in three growth stages (tillering, heading and grain filling) were recorded. Photosynthesis rate (P_N), stomatal conductance (g_s), transpiration rate (E) and intercellular CO_2 concentration (C_i) were measured using a portable open-system infrared gas analyzer (*LCi, ADC Bioscientific Ltd.*, Hoddesdon, UK).

All measurements were done in 10-12 a.m. and light intensity equivalent to 1200-1800 μmol photons.m^{-2}. It is reported that g_s during 10 am to 1 pm no significant changes (Clark and Mc ciag, 1982) and also at this light intensity g_s reaches to a maximum. Before measuring, apparatus started for 10 minutes. For measuring gas exchange parameters, same leaves of plants in each treatment placed in chamber glass clamp of apparatus. Data recorded after 45 seconds as the chamber conditions receive as stable state.

Photosynthetic water use efficiency (WUE) and Carboxylation efficiency (CE) also was calculated based on the following formula (Ashraf *et al*; 2002):

Photosynthetic water use efficiency (WUE_b) = P_N /E

Carboxylation efficiency (CE) = P_N /C_i

Chlorophyll content index (CCI)

Chlorophyll content index was measured by a chlorophyll meter handheld device (CCM-200 ADC, UK) in all three stages from 10 randomly selected plants'. Middle part of same leaves was used for this reason. In tillering fourth or fifth leaf was measured and in two later growths stages the flag leaves were measured.

Chlorophyll a, b and total:

Chlorophyll content of flag leaf in anthesis stage determined by method which described by Meidner (1984). The statistical analysis was done by using software MSTATC and SPSS. Means comparison was done by Duncan multiple test.

RESULTS AND DISCUSSION

Photosynthesis rate (P_N)

In both planting date and in all three growth stages priming significantly increased P_N rate. Except to grain filling stage, in tillering and heading stages early planted wheat had higher P_N compared to late plated wheat (Table 2, 3 and 4). The highest P_N in both planting date was observed in heading stage, then in tillering stage. In both planting dates, a decline was found in P_N in grain filling stage compared to heading stage (Table 3 and 4). In addition, at grain filling stage, all prim treatments in late planting had higher P_N compared to conventional planting date. It seems that, plants in the late planting treatment had younger tissues than conventional planting date. Also, it seems that, in grain filling compared to heading and tillering stages leaves was matured and more aged and therefore, had lower capacity in photosynthesis. In addition, it is possible in heading and pollination stage there was a higher demand for photoassimilates and may be it is a reason for the highest P_N in this stage. As mentioned above, priming with SA leads to increasing P_N. Among priming treatments, priming with 1200 µM concentration, in both planting date and in all three growth stages had the highest rate of photosynthesis (Table 2, 3 and 4).

Enhancement activity of carbonic anhydrase in leaves of mustard (Fariduddin *et al*. 2003) and Rubisco in maize (Khodary, 2004) by application of SA was reported. Also, protection of the photosynthetic apparatus has also been reported in SA treated tomato plants (Poor *et al*., 2011). On the other hand, higher concentrations of SA may have prevention effects on photosynthesis (Janda et al., 2014). Our results show that in concentrations over 1200 µM a decline observed in P_N.

Stomatal conductivity (g_s)

Seed priming increased g_s in three stages and in both planting dates. Also, it compensated the reduction in g_s in late planting in all growth stages. g_s in both planting and in the heading stage reached highest value in 1200 µM SA compared to other SA treatments (Table 2, 3 and 4). In comparison of two planting dates, g_s in late planting had higher value than conventional planting date in grain filling stage. Among growth stages the lowest g_s were observed in grain filling and highest in heading stage. The high ratio of g_s in heading compared to grain filling and tillering may be due to young age of leaves and demand of atmosphere for transpiration, respectively.

In contrast, in tillering stage as environment was cool than two other stages the g_s showed lower amounts. It is reported exogenous application of SA in wheat promoted growth and yield which associated with increased

photosynthesis capacity and g_s. (Arfan *et al*. 2007).

Transpiration rate (*E*)

The highest *E* was observed in the heading and the lowest in tillering and grain filling stages. Priming increased *E* in all different growth stages. Among priming treatments, 1200 µM had the most effects on *E* in both planting dates and all growth stages. In general, priming by SA improved the *E* values and enhanced its values compared to control treatments (Table 2, 3 and 4). Higher rate of transpiration may be related to increasing root development or efficient uptake of water by increasing root length and density which reported by Sandoval-Yepiz (2004) and Abdolahi and Shekari (2013). The lower value of *E* in tillering and grain filling stages may be due to cool temperature in early spring; and maturation of leaves, and therefore, reduction in capacity of leaves transpiration from aged plants, respectively.

Intercellular CO_2 concentration (C_i)

C_i was decreased by application of SA in three stages and two planting date and control treatments had higher *Ci* values. Among three growth stages the lowest C_i was found in heading stage and two other stages had higher values than mentioned stage (Table 2, 3 and 4). In general, 1200 µM of SA showed the lowest rate in all treatments expect tillering stage. In tillering stage 400 and 800 µM and in grain filling 400 µM of SA had not significant differences with control treatments. In tillering stage 2000 and 1600 µM SA in conventional and late planting had lowest *Ci* respectively (Table 2, 3 and 4). Lower rate of C_i in priming treatments than control treatments may due to higher CE or higher performance in assimilation of CO_2 in photosynthesis process. It was reported foliar spraying of 0.5-2.5 mmol.L^{-1} of SA on cucumber seedlings before the low temperature stress increased the leaf P_N, g_s, *E*, Φ PSII, Fv/Fm, while decreased the *Ci* (Liu *et al*., 2009).

Carboxylation efficiency (CE)

Priming significantly increased CE in all growth stages and two planting dates (Table 2, 3 and 4). The highest value of CE was observed in heading stage and conventional planting date and the lowest was observed in grain filling stage and conventional planting date. Since, the values of P_N were higher and *Ci* was lower in this stage, it is reasonably highest amounts of CE found in this stage. In heading stage, priming in highest values improved CE to 42% and 26% on the first and second planting date compared to control treatments, respectively. At all phenological stages, 1200 µM treatment, had the highest CE rate. High CE in priming treatments suggests more effective assimilation of carbon in these treatments relative to control treatments. Zhen *et al.* (2010) reported *Chrysanthemum* plants treatment with ASA increased CE, so caused reduction in C_i under low temperature stress with lower light intensity. They suggested that more tolerance to cold stress correlated with higher values of CE, g_s and P_N.

Photosynthetic water use efficiency (WUE)

The lowest rate of WUE was observed in heading stage and the highest was observed in tillering stage (table 2, 3 and 4).

Table 2. Effect of priming by salicylic acid (SA) on, net photosynthetic rate (PN), stomatal conductance (gs), Transpiration Rate (E), intercellular CO_2 concentration (Ci), Carboxylation efficiency (CE), and photosynthetic water use efficiency (WUE) in tillering stage in wheat plants.

Planting date	Priming [µM]	P_N [µmol (CO_2) $m^{-2}s^{-1}$]	g_s [mmol $m^{-2}s^{-1}$]	E [mmol(H_2O) $m^{-2}s^{-1}$]	Ci [µmol(CO_2) $mol(air)^{-1}$]	CE [mmol (CO_2) $m^{-2}s^{-1}$]	WUE [µmol (CO_2) $mmol^{-1}$(H_2O)]
Stress period							
Recommended planting date	Control	13.472^{bc}	175^{d}	3.572^{h}	233.5^{a}	57.82^{efg}	3.77^{a}
	400	15.403^{abc}	245^{c}	4.375^{cde}	233.5^{a}	65.98^{bcd}	3.52^{abc}
	800	16.712^{abc}	292.5^{b}	5.463^{a}	232.5^{a}	83.04^{a}	3.06^{abc}
	1200	18.155^{a}	325^{a}	5.84^{a}	216.75^{b}	83.175^{a}	3.396^{abc}
	1600	16.862^{a}	250^{c}	4.927^{b}	216.5^{b}	69.183^{bc}	3.058^{bc}
	2000	15.473^{bc}	202.5^{d}	4.33^{de}	185.833^{f}	55.763^{fg}	3.532^{abc}
	2400	15.028^{bc}	200^{d}	4.232^{efg}	200.75^{cd}	52.403^{g}	3.107^{abc}
Late planting	Control	12.335^{bc}	175^{d}	3.742^{gh}	234.75^{a}	63.675^{cde}	3.512^{abc}
	400	14.403^{abc}	181.667^{d}	4.239^{efg}	218.5^{b}	64.983^{cde}	3.462^{abc}
	800	15.048^{abc}	235^{c}	4.259^{ef}	206.75^{c}	70.745^{bc}	3.141^{abc}
	1200	17.092^{ab}	252.5^{c}	4.852^{bc}	204^{cd}	73.35^{b}	3.572^{abc}
	1600	14.955^{bc}	237.5^{c}	4.782^{bcd}	192.25^{ef}	65.983^{bcd}	2.888^{c}
	2000	13.523^{c}	202.5d	4.573^{bcde}	198.25^{de}	61.46^{def}	3.789^{ab}
	2400	12.35^{bc}	140^{e}	3.743^{fgh}	204.75^{cd}	54.62^{fg}	3.305^{abc}
		ANOVA					
date		**	***	***	***	n.s.	n.s.
Priming		n.s.	***	***	***	***	*
date× priming		**	***	***	***	***	*

* Within each column, different letters indicate significant differences at $P \leq 0.05$ (Duncan test). n.s., *, ** and *** indicate non-significant or significant differences at P, 0.05, 0.01 or 0.001, respectively.

Table 3. Effect of priming by salicylic acid (SA) on, net photosynthetic rate (PN), stomatal conductance (gs), Transpiration Rate (E), intercellular CO_2 concentration (Ci), Carboxylation efficiency (CE), and photosynthetic water use efficiency (WUE) in heading stage in wheat plants.

Planting date	Priming [µM]	P_N [µmol (CO_2) $m^{-2}s^{-1}$]	g_s [mmol $m^{-2}s^{-1}$]	E [mmol(H_2O) $m^{-2}s^{-1}$]	Ci [µmol(CO_2) mol(air)$^{-1}$]	CE [mmol (CO_2) $m^{-2}s^{-1}$]	WUE [µmol (CO_2) $mmol^{-1}$ (H_2O)]
Stress period							
Recommended planting date	Control	17.758^{gh}	345^{ef}	8.11^{cde}	238.5^{ab}	78.79^{hi}	1.945^{c}
	400	21.535^{bc}	382.5^{de}	8.565^{bcde}	225.733^{cd}	87.485^{defg}	2.259^{abc}
	800	22.187^{ab}	420^{cd}	9.77^{ab}	216.25^{de}	99.410^{c}	2.517^{a}
	1200	23.553^{a}	537.5^{b}	10.225^{a}	209.5^{e}	111.79^{a}	2.001^{c}
	1600	20.395^{bcd}	502.5^{b}	9.68^{ab}	210.5^{e}	108.79^{ab}	2.271^{abc}
	2000	19.897^{cde}	420^{cd}	8.735^{bcde}	225.25^{cd}	103.09^{bc}	2.111^{bc}
	2400	19.077^{defg}	332.5^{ef}	7.910^{de}	234.233^{abc}	84.095^{efgh}	2.305^{abc}
Late planting	Control	17.052^{h}	307.5^{f}	7.555^{e}	242.733^{a}	75.120^{i}	2.163^{abc}
	400	17.967^{fgh}	332.5^{ef}	8.978^{abcd}	218^{de}	78.226^{hi}	2.165^{abc}
	800	19.025^{defg}	465^{bc}	9.058^{abcd}	216.25^{de}	89.358^{def}	2.140^{bc}
	1200	20.672^{bcd}	622.5^{a}	9.58^{ab}	210.25^{e}	94.82^{cd}	2.278^{abc}
	1600	19.69^{def}	397.5^{cde}	9.207^{abc}	217.25^{de}	90.58^{de}	2.135^{bc}
	2000	18.150^{efgh}	355^{def}	8.497^{bcde}	218^{de}	81.025^{fghi}	2.419^{ab}
	2400	17.453^{gh}	327.5^{ef}	7.82^{de}	230.667^{bc}	80.36^{ghi}	2.232^{abc}
		ANOVA					
Date		***	n.s.	n.s.	n.s.	***	n.s.
Priming		***	***	*	***	***	*
date × priming		***	***	*	***	***	*

* Within each column, different letters indicate significant differences at $P \leq 0.05$ (Duncan test). n.s., *, ** and *** indicate non-significant or significant differences at P, 0.05, 0.01 or 0.001, respectively.

Table 4. Effect of priming by salicylic acid (SA) on, net photosynthetic rate (PN), stomatal conductance (gs), Transpiration Rate (E), intercellular CO2 concentration (Ci), Carboxylation efficiency (CE), and photosynthetic water use efficiency (WUE) in grain filling stage in wheat plants.

Planting date	Priming [µM]	P_N [µmol (CO_2) $m^{-2}s^{-1}$]	g_s [mmol $m^{-2}s^{-1}$]	E [mmol(H_2O) $m^{-2}s^{-1}$]	Ci [µmol(CO_2) $mol(air)^{-1}$]	CE [mmol (CO_2) $m^{-2}s^{-1}$]	WUE [µmol (CO_2) $mmol^{-1}$(H_2O)]
Stress period							
Recommended planting date	Control	7.53^{g}	77.5^{de}	3.547^{bc}	226.583^{a}	39.593^{g}	2.122^{bc}
	400	7.803^{fg}	80^{de}	4.368^{b}	214.417^{ab}	40.063^{fg}	1.79^{a}
	800	9.42^{ef}	82.5^{de}	4.723^{b}	206.75^{bc}	67^{bc}	1.788^{c}
	1200	11.232^{cd}	165^{b}	4.918^{ab}	187.75^{cde}	68.253^{bc}	3.416^{bc}
	1600	10.655^{de}	155^{b}	4.712^{b}	172^{ef}	56.16^{de}	2.383^{bc}
	2000	9.082^{efg}	102.5^{cd}	4.532^{b}	178.333^{ef}	48.77^{e}	3.142^{bc}
	2400	9.017^{efg}	102.5^{cd}	4.252^{b}	197.75^{bcd}	48.123^{ef}	2.283^{bc}
Late planting	Control	11.947^{bcd}	140^{b}	2.292^{c}	214^{ab}	61.9^{cd}	5.21^{bc}
	400	13.053^{b}	143.5^{b}	3.842^{b}	198.75^{bcd}	62.08^{cd}	2.267^{bc}
	800	13.334^{b}	160^{b}	4.342^{b}	185.25^{de}	76.623^{a}	2.716^{bc}
	1200	15.607^{a}	205^{a}	6.267^{a}	165^{f}	78.6^{a}	1.809^{c}
	1600	12.728^{bc}	147.5^{b}	4.855^{b}	170.25^{ef}	71.133^{ab}	3.468^{b}
	2000	12.305^{bcd}	110^{c}	3.745^{b}	184.167^{de}	63.663^{bcd}	2.215^{bc}
	2400	8.852^{fg}	67.5^{e}	3.572^{bc}	208^{ab}	32.14^{g}	2.479^{bc}
		ANOVA					
date		***	***	n.s.	*	***	*
Priming		***	***	**	*	***	**
date × priming		***	***	*	***	***	**

* Within each column, different letters indicate significant differences at $P \leq 0.05$ (Duncan test). n.s., *, ** and *** indicate non-significant or significant differences at P, 0.05, 0.01 or 0.001, respectively.

This increment may be due to more reduction in E than to P_N in this phenological stage because of cool temperature in tillering stage. With some exceptions WUE was decreased by priming (Table 2, 3 and 4). Exceptions was found in late planting treatments in tillering and recommended planting in heading. It seems priming by SA enhanced water status of plants and RWC (Abdollahi and Shekari, 2013). It is reasonable from E and g_s data which increased in SA treatments compared to control treatments. Therefore, transpiration rate increased by SA application more than P_N and WUE relatively decreased. Although, in our experiment WUE decreased by seed priming with SA, increasing in WUE by pretreatment with SA reported previously (Liu *et al*., 2011; Fariduddin *et al*., 2003; Khan *et al*., 2003).

Chlorophyll Index and Chlorophyll Content

In all growth stages first planting date had higher chlorophyll content index (CCI) compared to second planting date. Although, in first planting date heading stage had highest value among other phenological stages, but in late planting the highest amount of CCI was found in grain filling (Table 5). The lowest value among three growth stages was observed in tillering stage and second planted plants. In general, seed priming with 1200 µM of SA had greatest impact on increasing the amount of CCI in both planting date and in all three growth stages. The lowest values was observed control treatments and in late planting date.

Like CCI, chlorophyll a and b were affected by seed priming and planting date treatments (Table 5). The amounts of Chl a, b, total and a/b were increased through 1200 µM of SA treatment relative to control treatments in both planting dates. The amount of Chl b was increased to 10.5% through 2000 µM of SA compared to control treatment in late planting. Overall, the amounts of Chl a and b on the first planting date were higher than second planting date. However, priming through SA could discount the adverse effects of late planting and the amount of chlorophyll reduction. It seems that the effect of SA on biosynthesis and/or protection of chlorophyll a are more than chlorophyll b. Because the ratio of chlorophyll a/b in primed treatments in both planting dates are more than control treatments.

Chlorophyll content is important in maintenance of photosynthetic capacities (Jiang and Huang, 2001) and a key factor in determination of photosynthesis rate and dry matter production (dos Santos et al., 2013). Also, it is stated chlorophyll content is the most reliable parameter to estimate leaf growth and development (Albert et al. 2012). Gunes et al. (2007) reported in maize plants salt stress or application of SA had not significant effect on chlorophyll a, b and total content, but SA reduced carotenoids contentment. In contrast, Arfan et al. (2007) stated salinity decreased chlorophyll content of wheat, but SA increased chlorophyll content. Similarly, Sinha *et al*. (1993) pointed out that chlorophyll and carotenoid contents of maize were increased upon treatment with SA. Treatment with 500 µM SA for 24 h before exposure to chilling provided protection on Rubisco activity and chlorophyll content (Yordanova and Popova,

2007). It seems that this effect of SA on photosynthetic pigments depends to types of species, cultivar, method of SA application and its concentrations.

Total dry weight (TDW)

In comparison of two planting date, first planting had more dry weight compared to second planting date. With some exceptions, in all growth stages in treatments which had higher photosynthetic rates, higher accumulation of dry matter was found (Table 2, 3, 4 and 5).

Table 5. Effect of priming by salicylic acid (SA) on, Chlorophyll content index (CCI) in three stages, chlorophyll (Chl) (a), (b), (a/b), (a+b) content, and Biological Yield (BY) at harvest.

Planting date	Priming [µM]	CCI (Tillering)	CCI (Heading)	CCI (Grain filling)	Chl *a* [mg g^{-1}]	Chl *b* [mg g^{-1}]	Chl *a+b* [mg g^{-1}]	Chl *a/ b*	BY [g m^{-2}]
Stress period									
Recommended planting date	Control	16.740^{bc}	41.33^{cd}	39.703^{def}	0.8315^{e}	0.3762^{abc}	1.208^{ef}	2.273^{de}	1237^{de}
	400	17.748^{bc}	44.76^{ab}	41.715^{cd}	0.8543^{e}	0.2936^{ef}	1.148^{fg}	2.910^{ab}	1437^{bc}
	800	22.4^{ab}	45.533^{ab}	46.38^{b}	0.9573^{cd}	0.3913^{a}	1.349^{c}	2.447^{cd}	1572^{ab}
	1200	28.210^{a}	46.85^{a}	48.95^{a}	1.16^{a}	0.4032^{a}	1.564^{a}	2.875^{ab}	1641^{a}
	1600	26.922^{a}	46.313^{a}	41.163^{cd}	1.084^{b}	0.3843^{ab}	1.469^{b}	2.843^{abc}	1605^{a}
	2000	23.52^{ab}	43.32^{bc}	38.893^{ef}	0.9434^{d}	0.3030^{ef}	1.246^{de}	3.114^{a}	1549^{ab}
	2400	23.160^{ab}	39.537^{de}	36.07^{gh}	0.986^{cd}	0.331^{cdef}	1.317^{cd}	2.979^{ab}	1322^{cd}
Late planting	Control	5.342^{e}	28.53^{ef}	33.923^{h}	0.751^{fg}	0.3372^{bcde}	1.088^{gh}	2.228^{de}	969^{f}
	400	5.96^{de}	36.173^{ef}	34.2^{h}	0.7965^{ef}	0.3106^{ef}	1.107^{gh}	2.575^{bcd}	1237^{de}
	800	10.183^{cde}	37.903^{h}	39.83^{de}	0.7135^{g}	0.3211^{def}	1.035^{h}	2.226^{de}	1281^{d}
	1200	14.01^{cd}	39.36^{de}	43.105^{c}	1.031^{bc}	0.3234^{cdef}	1.355^{c}	3.203^{a}	1284^{d}
	1600	4.805^{e}	37.35^{ef}	41.533^{cd}	0.8415^{e}	0.2841^{f}	1.126^{fgh}	2.963^{ab}	1188^{de}
	2000	4.433^{e}	32.333^{g}	38.403^{ef}	0.747^{fg}	0.3726^{abcd}	1.120^{fgh}	2.008^{e}	1105^{ef}
	2400	4.09^{e}	31.46^{g}	37.603^{fg}	0.628^{h}	0.2911^{ef}	0.919^{i}	2.174^{de}	1096^{ef}
		ANOVA							
date		***	***	***	***	***	***	***	***
Priming		n.s.	***	***	***	**	***	***	***
date × priming		**	***	***	***	***	***	***	n.s.

* Within each column, different letters indicate significant differences at $P \leq 0.05$ (Duncan test). n.s., *, ** and *** indicate non-significant or significant differences at P, 0.05, 0.01 or 0.001, respectively

Increment in fresh and dry weight of plants by SA treatment may due to increase in cellular dividing rate in apical meristem of root and shoot of plants which enhance plant growth (Sakhabutdinova et al., 2003). Horvath et al. (2007) reported in wheat seedlings SA enhanced growth rate via increasing auxins and cytokinins concentrations. Also, Agami (2013) stated higher rate of dry matter production of maize plants both in normal and salt stress conditions by application of SA due to induction of antioxidant enzymes activities, proline and photosynthetic pigments. Increasing dry weight of artichoke plants by application of SA reported by Rajabi et al (2013) Fariduddin et al. (2003). On the other hand, Singh and Usha (2003) showed high concentrations of SA have preventive effects on wheat and maize growth. In our experiment seed priming with 2000 and 2004 μM SA concentrations had unfavorable effects on most recorded traits.

CONCLUSIONS

Presented results showed that change in planting date could affect photosynthetic parameters. In late planted plants PN was lower than conventional planting in tillering and heading stages, but in grain filling stage this trend was reversed. This trend approximately was found in gs and E traits. The highest values for PN, gs, E and CE were obtained in heading and then in tillering stages. In contrast, Ci was lower in heading stage and highest in grain filling stage. It seems that, in heading and flowering stage due to higher demand for photoassimilates PN showed higher values and in grain filling stage by aging of leaves capacity of photosynthesis relatively decreased. Seed priming by SA significantly increased PN and related parameters. Furthermore, the highest and lowest of WUE were achieved in tillering and heading, respectively. May be due to low temperature in early spring, the value of E was lower than other stages and this affect WUE in this stage. Seed priming with SA decreased WUE compared to control treatment in both planting dates. As shown by gs, it seems SA induced to more opening the stoma and therefore increment in E was more than PN. According to results, seed pretreatment with 1200 μM SA had appropriate performance than other SA concentrations.

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Vera POPOVIĆ, Ljubiša KOLARIĆ,
Ljubiša ŽIVANOVIĆ, Jela IKANOVIĆ,
Vera RAJIČIĆ, Gordana DOZET, Petar STEVANOVIĆ[1]

INFLUENCE OF ROW SPACING ON NAR–NET PHOTOSYNTHESIS PRODUCTIVITY OF *Glycine max* (L.) Merrill

SUMMARY

Soybean (*Glycine max* (L.) Merrill) is important legume which is used for humans diet and animal feeding. Beside the significance of certain agro-technical measures (choice of variety, sowing techniques), some physiological indicators, such as the net production of photosynthesis (NAR), are also important for yields achieving and evaluating. The study of the row spacing effects (20, 45 and 70 cm) on the productivity of photosynthesis of soybean varieties (Bosa, Balkan and Dragana) were carried out on the soil type of chernozem. The tendency of growth of surface area and number of leaves per plant was recorded up to the fourth measurement in the Bosa and Balkan varieties and until the fifth measurement in the Dragana variety, when the seed formation phase (R5) was established. The highest NAR was observed in early stages of growth, 10-25 days after emergence, while decreasing in later stages. The highest values of NAR in both examined years were recorded at square sowing (20 cm).

Keywords: soybean, variety, row spacing, productivity of photosynthesis

INTRODUCTION

Soybean - *Glycine max* (L.) Merrill is important legume which is used in Serbia because of its high yield, good quality of grain. The climate has a major effect upon plant growth and development, and often represents a limiting production factor. Soybean's importance comes first and foremost from the chemical composition of its grain, which is about 40% protein and around 20% oil. Because soybeans can be used whole or can be processed to obtain oil or protein, the plant is used widely and extensively not only in the food sector but in various other industries as well. Soybean meal is an indispensable source of protein in the nutrition of livestock, poultry and fish. We must not forget the role of soybean in field crop production either. Being a legume, soybean has the ability to fix atmospheric nitrogen and provide itself with sufficient amounts of

[1] Vera M. Popović, (corresponding author: vera.popovic@nsseme.com), Institute of Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad, SERBIA; Ljubiša Kolarić, Ljubiša Živanović, Jela Ikanović, University of Belgrade, Faculty of Agriculture, Nemanjina 6, Zemun Belgrade, SERBIA; Gordana Dozet, Megatrend University, Faculty of Biofarming, Bačka Topola, SERBIA; Vera Rajičić, Center for Small Grain, Kragujevac, SERBIA; Petar Stevanović, Inspection Affairs Administration of Republika Srpska, Banja Luka, BOSNIA AND HERZEGOVINA;
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readily available nitrogen, thus reducing the need for nitrogen fertilizer application. Leguminous plants, including soybean, are capable to form root nodules in symbiosis with bacteria of *Rhizobium* and *Bradyrhizobium* genera, and during that process they convert inorganic nitrogen (N_2) into ammonium ions (NH_4^+) available to the plant. *Bradyrhizobium japonicum* has a symbiotic relationship with legumes, or more specifically soybean plants. This makes this plant a very good fit for crop rotations (Hrustić et al., 1998; Simić and Vučković, 2014; Terzić et al., 2016; 2017; Kresović et al., 2016; Popović et al., 2013a; 2015; 2016). The introduction of new practices for increasing the seasonal productivity and forage quality of temporary swards by annual legume requires studies on plant material of different origin (Vasileva and Vasilev, 2012; Naydenova et al., 2014; Bozhanska et al., 2016).

Soybean is one of the most significant protein-oil plants. Beside the significance of certain agro-technical measures (such as sowing methods, the choice variety, plot, etc.) some physiological indicators, such as the Net Production of Photosynthesis (NAR), are also important for yields achieving and evaluating. The influence of these physiological indicators on yield and quality of soybean grain is different, according to results of numerous researchers.

A high and stable soybean yield can be achieved only when it is based on the cultivation of varieties of high genetic yield potential with the application of intensive agro-technology. Producers of soybeans in our country have a wide range of domestic varieties that are highly yielding, not genetically modified (Popović, 2010) and adapted to our climate.

One of the conditions for achieving high and stable yields is the proper arrangement of plants at appropriate crop densities. It is known that the square shape is an ideal vegetation space. However, in practice, it is difficult to achieve the shape of a square if soybean is sown at an intermediate distance of 50 cm and a distance of 3-5 cm.

By changing the shape of the vegetation space, ie. the intermediate distance, the microclimatic conditions of growth soybean plants is changes. Soybean plants is very sensitive with change of the microclimatic conditions, especially in the flowering stage. The shape of vegetation or sowing method has been studied in almost all areas of soybean cultivation. When sowing at a larger interstitial distance, much of the sunlight falls between rows and remains unused, especially in the early part of the soybean vegetation period (Kolarić, 2010). According to many studies, the applied crop density is the prerequisite for expressing the maximum genetic potential for the yield of these varieties (Bullock et al., 1998; Bowers et al., 2000; Holshouser and Whittaker; 2002; Heatherly et al., 2002; Nenadić et al., 2003; Vignjevic, 2006; Popović, 2016a).

The aim of this research is to study the influence of the size of the interstitial spacing at the same density of crops on the productivity of soybean photosynthesis. Based on the results it will be given recommendation for modern soybean technology.

MATERIAL AND METHODS

Research was carried out on the test-field in Zemun Polje, on the bad carbonate chernozem soil type, in period of two year. Micro-test was set up as a two-factor, split-plot method in four repetitions. The factors were: row spacing (A) (20, 45 and 70 cm) and variety (B): Bosa (0 maturity group), Balkan (I maturity group), Novi Sad; Dragana (II maturity group).

The density of crops within the variety was the same for all varieties and amounted to 500,000 plants per hectare for the Bosa, 450,000 plants per hectare for the Balkan variety and 400,000 plants per hectare for the Dragana variety. The size of calculated sample parcels was 5.4 m^2 (6.0 x 0.9 m) for a combination of sowing at 45 cm between rows, 6.0 m^2 (6.0 x 1.0 m) for a variation of sowing at 20 cm between rows and 8.4 m^2 (6.0 x 1.4 m) for planting at 70 cm spacing between rows, Kolarić et al., 2014a; 2014b. In the experiments, standard agro-technology for the production of soybean was applied, excluding studied factors. The soybean pre-crop, in both years of research, was corn. Deep plowing (25 cm) was done in the autumn immediately after maize harvesting, and on that occasion, 100 kg ha^{-1} UREA (46% N) was applied. Early ground preparation was carried out in the spring with seeders. Sowing was done on April 23th in the first examined year and on May 5th in the second examined year. The seed is directly inoculated with the microbiological preparation NS-Nitragin immediately prior to sowing. During the vegetation period, weed weeding and hoeing takes place on two occasions. Harvest was done manually on September 10th in the first and September 17th in the second examined year. During the vegetation period, the photosynthesis productivity was monitored starting from the phase of the first permanent leaves on the stalk until the beginning of the ripening of pods. These measurements were performed every 10 days by taking samples of 5 plants from each accounting plot and from all repetitions.

The surface of the samples was determined by measuring the maximum length and width of the leaves and multiplying with the correction factor 0.72 (Sarić et al., 1986). Based on surface area parameters, fresh and dry matter, NAR (net assimilation rate, net assimilation) was calculated:

$$N.A.R\ (g/m^2/day) = \frac{W_2 - W_1}{A_2 - A_1} \times \frac{LnA_2 - LnA_1}{T_2 - T_1}$$

Where in:

W1 = initial weight dry weight
W2 = final dry matter weight
T1 = initial harvest time
T2 = final harvest time
A1 = initial fresh leaf weight
A2 = final fresh leaf weight
Ln = natural logarithm ($\log_e$)

Soil Conditions

According to the pedological study of the soil in Zemun Polje, carbon chernozem is characterized by humus –accumulative (Ah) horizon of 0-50 cm of depth, dark block color and powdery clay agril texture (Šarčević-Todosijević et

al., 2016). Study area was conducted two year period in Zemun Polje, (latitude: 44º 52´00´´ N and longitude: 20º 19´ 00´´ E) region of Serbia on the chernozem soil type (pH = 7.95).

Table 1. Chemical properties of soil, Zemun Polje, Serbia

Depth (cm)	pH in		$CaCO_3$ (%)	*Humus* (%)	*Total N* (%)	C/N	*Accessible N* (ppm)			*mg/100gr soil*	
	H_2O	KCl					NH_4	NO_3	NH_4+NO_3	P_2O_5	K_2O
0-20	7.95	7.25	1.6	2.87	0.180	9.2:1	8.4	15.4	23.8	26.8	25.4
20-40	8.00	7.40	2.2	2.72	0.175	9.0:1	4.9	16.8	21.7	26.8	24.3

The soil was neutral to weak alkaline reaction, well provided by total nitrogen and humus, poorly carbonate and with an increased content of P_2O_5 and K_2O (Tab. 1).

RESULTS AND DISCUSSION

Meteorological conditions

Meteorological data were obtained from the Meteorological Station, Zemun Polje, Serbia (Figure 1). Weather conditions are variable and unpredictable (Popović et al., 2013a; 2013b; 2014; 2015; 2016b; Kolarić et al., 2015a; 2015b; Mandić et al., 2016). In first year, about 2°C higher air temperatures were recorded, compared to second year, where temperatures were at the level of perennial averages (Figure 1).

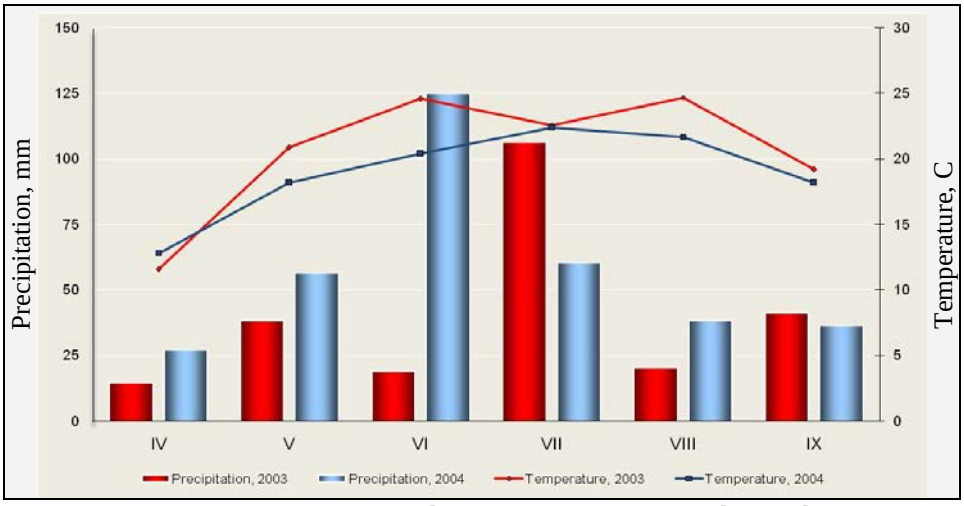

Figure 1. Precipitation and temperature, Zemun Polje, Serbia

In second year, the amount of precipitation during the vegetation period was at the level of perennial averages and about 105 mm higher compared to first year (Figure 1).

The influence of the row spacing and genotype on the Net Photosynthesis Productivity (NAR)

The productivity of photosynthesis represents the increase in dry matter per unit area of the leaf surface in the unit of time (Kojić, 1987). Productivity of photosynthesis of one species does not have a constant value and, among other things, depends on external conditions (temperature, humidity, light intensity).

This parameter expresses the increase in dry weight. Therefore, this parameter was called pure or Net Photosynthesis Productivity (Popović, 1976; Haloi and Balden, 1986; Kojić, 1987; Kastori, 1989).

During the growing period of the genotypes encompassed by studies, NAR was uneven with different tendencies in both years of research (Tables 2 and 3).

Based on the research, we conclude that the productivity of photosynthesis of one species does not have a constant value, but depends on climatic conditions (temperature, humidity, etc.). Precipitation quantity during soybean growing period in first year was 240 mm and in second year was 350 mm, Figure 1.

Table 2. Influence of row spacing and variety on the net assimilation in first year (NAR g/m^2 / day)

***Row spacing* (A)**	***Variety* (B)**	**Interval**						
		I-II	**II-III**	**III-IV**	**IV-V**	**V-VI**	**VI-VII**	**VII-VIII**
20 45 70	Bosa	3.30 2.83 2.72	4.12 2.92 2.51	1.50 1.05 0.98	1.36 0.64 0.18	0.92 0.24 0.16	-	-
Average		2.95	3.18	1.18	0.73	0.44	-	-
20 45 70	Balkan	3.40 2.73 2.59	3.07 2.91 2.77	3.08 2.91 2.71	1.19 1.20 1.12	0.86 0.45 0.36	0.27 0.16 0.22	-
Average		2.91	2.92	2.90	1.17	0.56	0.22	-
20 45 70	Dragana	3.23 3.02 2.56	3.32 2.71 2.09	3.20 2.71 2.35	2.36 1.81 1.64	0.50 0.45 0.24	0.64 0.57 0.41	0.23 0.12 0.06
Average		2.94	2.71	2.75	1.94	0.40	0.54	0.14
Average	20 45 70	3.31 2.86 2.62	3.50 2.85 2.46	2.59 2.22 2.01	1.64 1.22 0.98	0.76 0.38 0.25	0.46 0.37 0.32	0.23 0.12 0.06
Total / Average		2.93	2.94	2.28	1.28	0.46	0.38	0.14

In favorable second year, similar to first, the highest NAR values were in the first two measurement intervals (on average, 3.19 and 2.61 g/m^2/day), Figure 2 and 3. However, later, unlike the previous year, there is a steady decrease in the value of this parameter. The lowest values of this parameter were determined at the end of the vegetation period for the three investigated varieties. In both years, the highest NAR values were recorded by sowing in narrow rows.

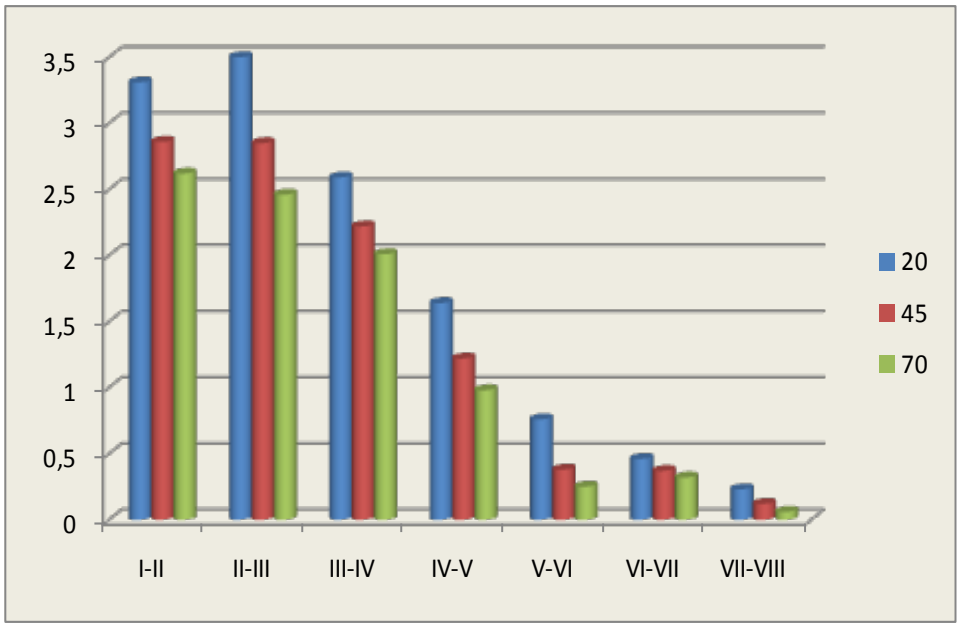

Figure 2. Influence of row spacing on the net assimilation the first year (NAR g/m^2/day)

The variety of the longer vegetation period had a higher net assimilation compared to the earlier variety.

It is a derived parameter which has based on the leaf surface and a formed dry mass at a certain time and all the effects on them are also reflected on the NAR, similar to that of garlic (Castillo et al., 1996; Rizzali and Villalobos, 2002). The size of the leaf surface and its duration significantly influence the yield level (Kastori, 1989).

The highest NAR was observed at early stages of growth, 10-25 days after emergence, while in later stages it was decreased. The NAR is the most appropriate form of displaying the photosynthetic effect of plants and crops.

In a 14 soybean varieties research, Mitrović (1958) found that the leaf surface was in a positive correlation with the yield of the grain per unit area.

Hanway and Weber (1971) found that, in the flowering stage, the total dry weight of the plants is consisting of leaves (55%), leafy stems (14%) and stalk (31%). The maximum dry matter of the leaf was obtained in the full blossoming phase. The maximum accumulation of total dry matter is achieved within 108 to

115 days after sowing both, determinant or indeterminate type of soybean, according to Egli and Legget (1973).

Table 3. Influence of row spacing and variety on the net assimilation in second year (NAR g/m^2 / day)

Row spacing (A)	*Variety* (B)	Interval							
		I-II	II-III	III-IV	IV-V	V-VI	VI-VII	VII-VIII	VIII-IX
20	Bosa	3.70	3.41	2.20	2.03	1.24	0.96		
45		2.85	2.49	2.16	1.81	0.88	0.71	-	-
70		2.59	1.85	1.94	1.68	0.72	0.29		
Average		3.04	2.58	2.10	1.84	0.85	0.75	-	-
20	Balkan	3.10	3.10	2.53	1.50	1.47	1.35	1.16	
45		3.09	2.51	2.43	1.31	1.39	1.10	0.82	-
70		3.05	2.29	1.79	1.29	1.39	1.04	0.34	
Average		3.08	2.63	2.25	1.37	1.42	1.16	0.77	-
20	Dragana	3.89	2.97	2.30	1.93	0.93	1.23	1.04	0.91
45		3.85	2.89	1.97	1.43	0.79	1.07	0.81	0.57
70		2.58	1.97	1.82	1.29	0.70	0.88	0.79	0.17
Average		3.44	2.61	2.03	1.55	0.81	1.06	0.88	0.55
Average	20	3.56	3.16	2.34	1.82	1.21	1.18	1.10	0.91
	45	3.26	2.63	2.19	1.52	1.02	0.96	0.82	0.57
	70	2.74	2.04	1.85	1.42	0.94	0.74	0.57	0.17
Total / Average		3.19	2.61	2.13	1.59	1.06	0.96	0.83	0.55

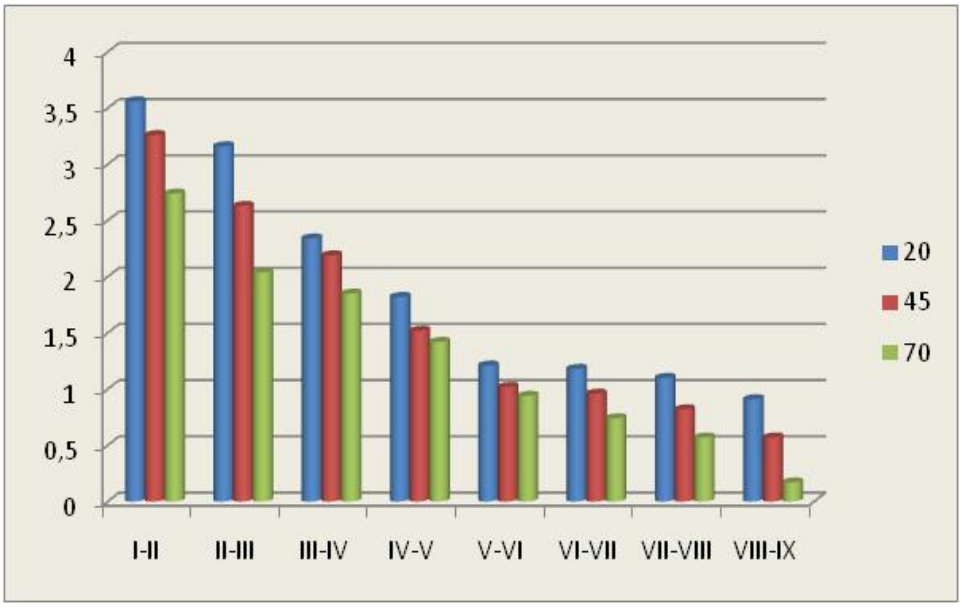

Figure 3. Influence of row spacing on the net assimilation in the second year (NAR g/m^2 / day)

However, in terms of the accumulation of vegetative mass, the variety did not differ significantly, since its maximum weight was achieved approximately 89 days after sowing. After that, it fell because the leaves dying out were faster than the growth of new ones. The value of net assimilation varies depending on the genotype, Kolomejchenko (1982) points out.

It was established that high productive wheat genotypes (7-9 t ha^{-1}) with the optimal architectonics possess higher rate of CO_2 assimilation during the leaf ontogenesis. Along with the high rate of photosynthesis, high values of photorespiration are characteristic for the high productive genotypes. Genotypes with moderate (4-5 t ha^{-1}) and low (3 t ha^{-1}) grain yield are characterized by relatively low rates of both CO_2 assimilation and photorespiration. A value of photorespiration constitutes 28-35% of photosynthetic rate in contrasting genotypes (Aliyev, 2012).

Bunce (2014) was tested whether soybean photosynthesis at high light and elevated CO_2 (ambient + 180 μmol mol^{-1}) was limited by electron transport (*J*) in FACE systems but by ribulose-bisphosphate carboxylation capacity (V_{Cmax}) in OTC. FACE systems with daytime and continuous CO_2 enrichment were also compared. The results indicated that in both cultivars examined, midday photosynthesis at high light was always limited by V_{Cmax}, both in the FACE and in the OTC systems.

The correct selection of the interstitial spacing affects the aging of a higher biomass of soybean and therefore productivity (Kolarić et al., 2014).

Constant climate changes force legume, forage crops and mixtures to adapt to the altered conditions and require better efficiency of resource utilization by mixtures. At present, the main interest is directed towards more drought resistant and drought tolerant components. Legume species that can provide self - sowing and persist continuously in the sward become of practical importance (Vasileva et al., 2015).

Leguminous plants maintain a very privileged relationship with the rhizosphere. Their agronomic interest results first of all from their ability to establish a mutualistic symbiosis with bacteria belonging to *Rhizobia* family for the utilization of atmospheric nitrogen as a nitrogen source (Giri and Joshi, 2010). It is estimated that approximately 40-60 million metric tons of atmospheric nitrogen is fixed by cultivated legume plants annually (Mansouri and Kheloufi, 2013). Indeed, this symbiosis allows them to produce in abundance proteins even in the absence of nitrogenous fertilization. They play consequently a key role in the crop rotation (Popovic, 2010).

The use of biomass for bioenergy creates new business opportunities in agriculture sector. Bioenergy production can significantly contribute to the development of rural areas and encourage creating new supply chains for biomass feedstock. The creation of new non-food markets for biomass could provide alternative income sources for farmers (EC 2012) Agricultural residues, may act as important source of renewable energy. However, despite progress in

these segments of crop production, the greatest potential for the use of biomass as fuel is seen in field (Čurović et al. 2016).

CONCLUSIONS

Based on the research, it can be concluded:

- Sowing at different intermittent distances has affected the parameter net productivity of photosynthesis of soybean varieties of different lengths of the vegetation period.
- The results show that the NAR increased until the fourth measurement, R_4, at the varieties: Bosa and Balkan, and at the fifth measurement, R_4, at variety Dragana when the seed formation phase (R_5) was established.
- In the conditions of the arid and semi-arid climates, to whom northern parts of our country mostly belong, the advantage in the planting structure of the areas under the soybean should be given to medium-early varieties, with respect to the optimal sowing period (until April 10), and by this to try to avoid the adverse environmental conditions that were frequent in the summer months (June and July), especially in the last decade.
- Significantly higher productivity of soybean photosynthesis can be achieved at smaller intervening distances (20 cm). In this way, a more favorable arrangement of plants, ie. shape of vegetation space is provided.

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Igor ĐURĐIĆ, Vladeta STEVOVIĆ, Vesna MILIĆ,
Tanja JAKIŠIĆ, Branka GOVEDARICA,
Milan JUGOVIĆ, Siniša BERJAN [1]

FORAGE PEA YIELD IN DIFFERENT AGROECOLOGICAL CONDITIONS

SUMMARY

Since the production area of forage peas is begin to increase, the experiment was set up at two localities, Banja Luka and East Sarajevo. For experiment was used five domestic varieties Saša and NS Junior from Serbia. The experiment was carried out with three types of fertilization (without the use of mineral fertilizers, basic fertilization with NPK and basic fertilization by NPK + nitrogen addition). The variety Saša had the highest yield of green mass and hay. The application of basic fertilization had the influence on tested parameters, while the application of basic fertilizer in combination with topdressing had poorer results. Agro-ecological conditions had the influence on yield of hay, so greater results are achieved on experimental field East Sarajevo.

Keywords: forage peas, yield, hay, green mass, variety

INTRODUCTION

Peas are usually used in the diet of humans and domestic animals, but it also playing an important role in fertilization as green fertilizer. According to Eric et al. (2007), peas are cultivated in the world at 7.621.928 ha, out of which 1.046.011 ha or 13.71 % is covered with forage pea. In our country pea is grown on small surfaces. Livestock peas can be used as green fodder, hay, silage, shade, for dehydration and if necessary green manure fertilizer (sideration). Pea's production is mainly focused to the production of grains, which represent concentrated animal feed. In our mountain-hilly production conditions, the production of hay is dominant, while silage production is less represented. Peas are sown mixed with cereals, mostly as early crops. Peas for the production of hay are little used, because it should be cutting in May, but May is a month with plenty of precipitation and it is difficult to dry a large mass of hay and that can affects the quality of hay which decreasing significantly. Early peas for the production of hay is cutting slightly later than the winter pea variety, when plants are in the stage of full flowering and the start of filling (Gatarić et al., 2014).

[1]Igor Đurđić, Vesna Milić (corresponding author: vesnamlc@yahoo.co.uk), Tanja Jakišić, Branka Govedarica, Milan Jugović, Siniša Berjan, University of East Sarajevo, Faculty of Agriculture, Vuka Karadjica 30, BOSNIA AND HERZEGOVINA, Vladeta Stević, Agronomic Faculty Čačak, University of Kragujevac, SERBIA

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

The yield of green mass in pure crop per hectare is 25-35 t, and in the mixture with cereals up to 50 t ha^{-1} (Mišković, 1986). In the research for the production of forage (Gatarić et al., 2014), peas is fertilized with combined NPK fertilizers, with: 40-80 kg ha-1 nitrogen, 80-100 kg ha^{-1} potassium and 80-120 kg ha^{-1} phosphorus. For the production of the early forage pea, it is recommended to fertilize with NPK (10:20:30) for lighter soil (Lončar et al., 1989, cited by Rapčan et al., 2004), and for the heavy soil with NPK (8:26:28), while Brkić (2002) recommended 40-150 kg ha^{-1} N entering a quantity that will satisfy pea needs. For the yield of the early spring pea, amounts of rainfall in May and the first decade of June (Kovac, 1994; Rapcan et al, 2006) are very important. Since early spring pea is grown from March to July in our agroecological conditions, the negative influence of high summer temperatures occurs during the development of pods (Duthion & Pigeaire, 1991). Temperatures above 26 °C in the stage immediately after flowering can significantly reduce the yield of grain due to decay of flowers (Popović et al., 2002).

MATERIAL AND METHODS

The experiment was set up in 2016 at the experimental field of the Agricultural Institute of Republic of Srpska in Banja Luka (altitude 160 meters) and the experimental field of the Faculty of Agriculture in East Sarajevo (altitude 550 m). The field experiment was set in a randomized block design with four replications. The sowing was done at 12.5 cm spacing and space of 8 cm in the main plot of 5 m^2. Seed of two varieties of fodder peas was used for sowing: NS Junior and Saša. In the study of the influence of mineral nutrition on the yield of green pea mass (t ha^{-1}) and yield of hay (t ha^{-1}),together with the control variant, the basic fertilizer application of 350 kg ha^{-1} $N_8P_{24}K_{24}$ and a variant with basic fertilization of 350 kg ha^{-1} $N_8P_{24}K_{24}$ + yield with 27 kg ha^{-1} (27% KAN used) were used. In Banja Luka, sowing was done on April 15 and harvest on July 15, while in East Sarajevo sowing took place on April 8, and harvest on July 18. At the floral stage, samples of the above ground mass of plants from a surface of 1 m^2 were taken from each repetition. After weighing, the mass of the samples was calculated on the quantity of above ground vegetative mass in t ha^{-1}. Then the drying of the sample was performed to obtain an air dry weight. Than the sample was weighed and conversion was performed to obtain the yield of hay.

The obtained data were processed by the method of variance analysis (MANOVA) and tested by the LSD test (STATISTICA 7.1 for Windows (Stat Soft 2005).

Agroecological conditions

The disposition of total monthly precipitation and average monthly air temperature for 2016 and perennial average for Banja Luka and Sarajevo is shown in Table 1. The temperature conditions for pea development were favorable. Average monthly air temperatures at both sites during the testing year were more than the perennial average; only in May 2016 the mean monthly temperature was somewhat lower than the perennial average. During the

vegetation period, precipitation oscillated from month to month. In 2016, in Banja Luka area, the extreme month was May whit only 0.5 mm of precipitation. Uneven precipitation patterns affect the forage pea yield.

Table 1. Average monthly air temperatures (°C), Monthly precipitation (mm) and Perennial averages for Banja Luka and Sarajevo

Month			I	II	III	IV	V	VI	VII
Banja Luka	2016.	Temperature (°C)	2.3	7.6	8.0	13.5	16.2	21.5	23.3
		Precipitation (mm)	109.7	108.5	122.2	0.5	100.6	117.8	125.9
	Average 1981-2010	Temperature (°C)	0.6	2.3	6.8	11.5	16.5	19.8	21.8
		Precipitation (mm)	69.7	59.1	87.5	84.3	89.4	112.4	81.4
Sarajevo	2016.	Temperature (°C)	1.2	7.4	7.1	12.9	13.9	19.5	21.1
		Precipitation (mm)	46.6	87	131.7	60.5	82.1	96.4	104.5
	Average 1961-1990	Temperature (°C)	-0.8	1.7	5.5	10	14.8	17.7	19.7
		Precipitation (mm)	74	63	73	76	85	94	83

The land on which the trials were performed in Banja Luka is of good physical characteristics with a depth of the plowed land up to 35 cm. According to agrochemical analysis done at the Faculty of Agriculture in East Sarajevo, plowed land contains 0.13% nitrogen, 8.6% calcium, 2.05% humus, 4040 mg / 100 g phosphorus and 38.48 mg / 100 g potassium. The soil reaction is neutral, the pH in nKCl is 6.97, while the experiment field of the Faculty of Agriculture in East Sarajevo contains 0.27% nitrogen, 4.12% humus, 4040 mg / 100 g phosphorus and 36.41 mg / 100 g potassium. The soil reaction is slightly acidic, the pH in nKCl is 6.39.

RESULTS AND DISCUSSION

The average yield of green mass was 29.7t ha^{-1} (Table 2). According to *Mišković* (1986) yields of green mass in pure crop per hectare is in the range 25-35 t, while in the mixture with cereals is up to 50 t. *Hoffman and Dér* (2003) found green mass yield of 29,68 and 33,03 t ha^{-1} in two pea genotypes, which is in accordance with our results.

The highest yield of green matter was recorded by variety Saša from Banja Luka (34,19 t ha-1) and it was significantly higher compared to the other tested variety, NS Junior (25,15 t ha-1). In control variant the yield of the green mass was 24.4t ha^{-1}. In case where basic fertilization was applied, the yield was 32.3 t ha^{-1}, and in case of basic fertilization +addition, it was 32.4 t ha^{-1}. Mineral

fertilization had a significant influence on the yield of green matter compared to the control variant. In the variant basic fertilization + addition, yield of green mass was lover comparing to variant with only basic fertilization. The location did not have influence on the yield of green mass and it was in the ranged from 29.2 t ha^{-1} to 30.1 t ha^{-1}.

Table 2. Yield of green mass (t ha^{-1}) / for forage

NPK quantity	Variety	Site		Average
		East Sarajevo	Banja Luka	
Control	NS Junior	17.4	21.5	19.5
	Saša	33.7	24.7	29.2
	Average	25.6	23.1	24.4
Basic fertilization	NS Junior	29.7	27.2	28.5
	Saša	37.0	35.2	36.1
	Average	33.3	31.2	32.3
Basic fertilization +supplementation	NS Junior	29.6	25.4	27.5
	Saša	33.4	41.0	37.2
	Average	31.5	33.2	32.4
Average	NS Junior	25.6	24.7	25.2
	Saša	34.7	33.7	34.2
Total average		30.1	29.2	29.7

Treatments	F-test	LSD 0,05	LSD 0,01
A (variety)	0.002	5.37	7.22
B (fertilization)	0.027	6.58	8.84
C (location)	0.727	5.37	7.22
AxB (variety x fertilization)	0.933	9.30	12.50
AxC (variety x location)	0.974	7.60	10.21
BxC (fertilization x location)	0.770	9.30	12.50
AxBxC (variety x fertilization x lcation)	0.173	13.16	17.68

The average yield of hay was 7.50 t ha^{-1} (Table 3). The highest yield of hay was recorded on Sasa varieties (8.61 t ha^{-1}), and it was significantly higher than NS Junior (6.39 t ha^{-1}). This location had significant influence on the yield of hay and it was in the ranged from 7.75 t ha-1 in East Sarajevo to 7.25 t ha^{-1} in Banja Luka. In the control variant, the yield of hay was 6.24 t ha^{-1}. In the variant basic fertilization, the yield was 8.18 t ha^{-1}, while in the variant basic fertilization + addition, was 8.09 t ha^{-1}. Comparing to control variant, application of different types of fertilization, had statistically significant influence to the increase of yield of hay.

Mihailović and Mišković (1988) obtained in their experiments the hay yield of 4.7-6.7 t ha^{-1}, while experiments of *Henneberg et al*. (1989) during three year trial found that hay yield depended on genotype and ranged from 1.82 to 9.80 tha^{-1}. Three year experiments of *Tekeli and Ates* (2003) showed average hay

yields in five pea lines of 3.44-7.38 t ha^{-1}. Similar results were obtained by *Ayaz et al.* (2004). Our results are in accordance with the results of the quoted authors.

Table 3. Hay yield (t ha^{-1}) / for forage

NPK quantity	Variety	Site		Average
		East Sarajevo	Banja Luka	
Control	NS Junior	4.46	5.52	4.99
	Saša	8.65	6.34	7.49
	Average	6.55	5.93	6.24
Basic fertilization	NS Junior	7.62	6.58	7.10
	Saša	9.49	9.03	9.26
	Average	8.55	7.81	8.18
Basic fertilization +supplementation	NS Junior	7.59	6.60	7.09
	Saša	8.68	9.51	9.09
	Average	8.14	8.05	8.09
Average	NS Junior	6.56	6.22	6.39
	Saša	8.94	8.28	8.61
Total average		7.75	7.25	7,50

Treatments	F-test	LSD 0,05	LSD 0,01
A (variety)	<.001	0.2011	0.2702
B (fertilization)	<.001	0.2464	0.3310
C (location)	<.001	0.2011	0.2702
AxB (variety x fertilization)	0.210	0.3484	0.4681
AxC (variety x location)	0.281	0.2845	0.3822
BxC (fertilization x location)	0.032	0.3484	0.4681
AxBxC (variety x fertilization x lcation)	0.791	0.4927	0.6619

CONCLUSIONS

Based on the obtained results, the following can be concluded:

- The highest yield of green mass had the variety Saša, while the lowest had variety NS Junior. The largest yields of hay had the varieties NS Junior and Saša, while the lowest had variety NS Junior.

-In the control variant, was recorded the smallest yields of green mass and hay. In the variant basic fertilization with NPK was recorded the best results. Thus, basic fertilization+ addition had poorer results compared to basic fertilization, in order to reduce pollution and save the environment, this measure can be omitted.

-Ecological conditions have influence on the yield of hay, while they have not affected the yield of green matter. In agro-ecological conditions in East Sarajevo, the yield of hay was significantly higher compared to the tested characteristic in the agro-ecological conditions of Banja Luka.

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Șerban CHIVULESCU, Ștefan LECA,
Diana SILAGHI, Valentin CRISTEA [1]

STRUCTURAL BIODIVERSITY AND DEAD WOOD IN VIRGIN FORESTS FROM EASTERN CARPATHIANS

SUMMARY

Owing to the high rate of global industrialization, widely distributed forest areas have decreased. Few of these natural forests have succeeded to remain untouched by human activities. The primary ecosystems are characterized by advanced age, high biodiversity and climax condition. These virgin forest, are found in Buzau Mountains, in the Eastern Carpathians of Romania. For understanding and develop the functional principles of virgin forests, field information was collected from three permanent research plots of one hectare area.

Gini index (G = 0,68 – 0,84) and Camino index (H = 1,62 – 1,74) were recorded for all permanent study plots. The obtained values reveal high heterogeneity. Total volume of dead wood is between 54,93 $m^3 \bullet ha^{-1}$ (Șapte Izvoare) and 123, 34 $m^3 \bullet ha^{-1}$ (Penteleu – Viforâta 2), most of it came from coniferous species (fir and spruce). There have been analyzed the relationships between dead wood and alive components using different statistical distribution functions (Beta, Gamma, Weibull), and the quantity of dry biomass and CO_2 stock from dead wood was estimated.

Keywords: virgin forests, structural biodiversity, Carpathians, dead wood CO_2 stock.

INTRODUCTION

Owning to the high rate of global industrialization widely distributed forest areas has been diminished. Few of these natural forests have succeeded to remain untouched by human activates. Natural ecosystems which have not been influenced by human activities, like virgin stands, have a more complex structure due to their natural dynamics (Hett and Loucks, 1976, Valbuena et al., 2012). The virgin forests present in Romanian Carpathians have a significant importance for the entire Europe, because they keep the natural values which have been lost from other countries' heritage and are very important in protecting, maintaining and monitoring the biodiversity (Ozcelik, 2009).

[1]Șerban Chivulescu (corresponding author: serban.chivulescu@gmail.com), National Institute for Research and Development in Forestry "Marin Drăcea" and Institute of Biology of Romanian Academy (SCOSAAR), ROMANIA; Ștefan Leca, Diana Silaghi, National Institute for Research and Development in Forestry "Marin Drăcea", ROMANIA; Valentin Cristea, Faculty of Silviculture and Forest Engineering / Transilvania University of Brasov, ROMANIA

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

An important aspect in stand evolution is the structural biodiversity (Roibu et al., 2008), that is why this element is vital in the research and understanding of the virgin forests functionality.

For describing the biodiversity of stands were used Gini and Camino indexes. Gini index was developed for economical purposes (Sen, 1973, Rouvinen and Kuuluvaien, 2005), but it was also used in ecological studies (Weiner, 1985, Knox et al., 1989, Nilsson, 1994, Stocker, 2002), being the most representative index for structural diversity (Roibu et al., 2008) due to its strictness (Cenuşă et al., 2002, Lexerod and Eid, 2006, Roibu et al., 2008).

Dead wood has been recognized to have an important ecological function as a resource for the ecosystems, structural components of the forest and in providing resources and habitats for a wide range of organisms (Ferris and Humphrey, 1999, Saniga and Saniga, 2004, Schuck et al., 2005, Humphrey et al., 2005, Merganičová and Merganič, 2010, Tomescu et al., 2011, Seibold et al., 2015). Dead wood also plays an important role in absorbing carbon (Kueppers et al., 2004, Merganičová and Merganič, 2010) and by this in slowing down the process of global warming.

MATERIAL AND METHODS

Study area

Research areas are situated in Southern Carpathians, in Romanian Curvature Mountains, more specific in Penteleu Mountains which are a subdivision of Buzău Mountains (Figure1). One plot has been installed in 2014 (Penteleu – Viforata 2) and the other two were installed in 2015 (Penteleu – Viforata 1 and Sapte Izvoare). All plots are characterized by high naturalness and they have relevant criteria's to enclose these forests in virgin forest category from the point of view of Romanian Environmental and Climate changes Ministerial Order 3397/2012 regarding the selection and identification of virgin and cvasi-virgin forests.

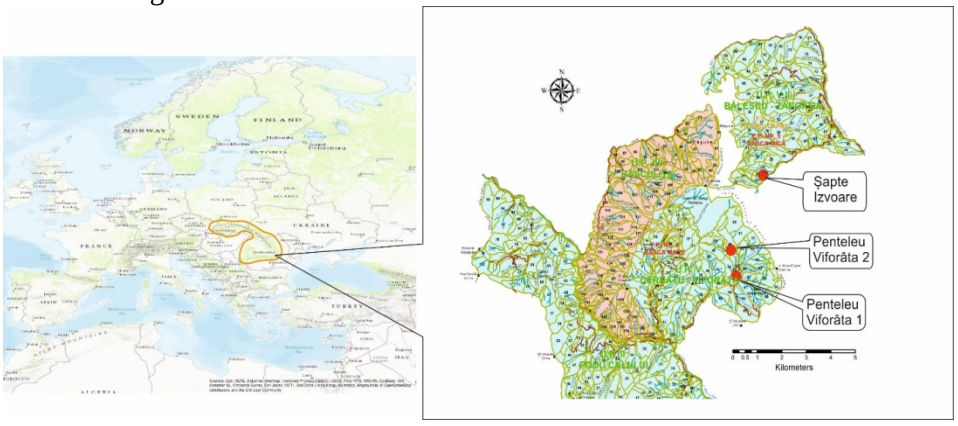

Figure 1. Permanent research plots from Curvature Carpathians (Natural Earth, Esri, ICAS)

Research method

Each one of the three researched plots has an area of one hectare - one having a circular shape (Penteleu – Viforâta 2) and the other two having a rectangular shape (Penteleu – Viforâta 1 and Șapte Izvoare). All live trees with diameter at breast height (dbh) higher than 80 mm were measured and the main dendrometrics characteristics (dbh, height, cenotic class, quality class) were registered. Standing dead wood with dbh higher than 8 cm and lying dead wood with top diameter higher than 8 cm were also measured and sampled (Table 1). Standing dead wood volume was calculated using the following regression equation:

$$\log v = a_0+a_1\log d+a_2\log^2 d+ a_3\log h +a_4\log^2 h$$

Where: h – height of standing dead wood; d – dbh; v- volume of standing dead wood; a_0, a_1, a_2, a_3, a_4 - regression coefficients (Giurgiu, Decei, Drăghiciu, 2004).

Lying dead wood volume was determined using Huber formula:

$$v = 0.785 \cdot l \cdot \sum d_i.$$

Table 1. Sampling dead wood

Dead wood category	Description	Notation
Snag 1	Standing tree died recently, with the majority of the branches intact or beginning to break, and intact or almost intact bark and intact top	S 1
Snag 2	Standing tree, died a while ago, without top and branches and also without bark or less bark remained	S 2
Log 1	Lying dead wood, still solid, with bark or beginning to lose bark	L 1
Log 2	Lying dead wood, soft and without bark	L 2

Descriptive statistics was made with help of PASTECS (Grosjean & Ibanez, 2014) package from RStudio program. Dry biomass and CO_2 was sampled for each category of dead wood. For standing dead wood, volume was transformed in biomass using the relation:

$$DB = V \bullet WD \text{ (Goslee et al., 2014)}$$

Where:DB is dry biomass; WD is wood density

WD was taken from wood density Romanian tables (Giurgiu, Decei, Drăghiciu, 2004) and was applied a reduction density factor according to the level of deterioration (UNFCC, 2013). Thereafter DB was transformed into CO_2 stock (C_{SDW}) using the relation:

$$C_{SDW} = DB \bullet CF \text{ (Goslee et al., 2014)}$$

Where: CF - carbon fraction usually used in the literature (IPCC 2006) GL,V4, CH4, (Table 4.3) with the value of 0,47 t/ha.

The relation used to determine dry biomass from lying dead wood was:

$$BM = V \bullet Dc \text{ (Goslee et al., 2014)}$$

Where:BM represents the dry biomass; V is volume of lying dead wood; Dc is medium density class (Walker et al., 2012).

Lying dead wood carbon stock (C_{LDW}) was determined using a relation which is composed of dry biomass (BM) and carbon fraction (CF):

$$C_{LDW} = BM \bullet CF \text{ (Goslee et al., 2014)}$$

Where: CF value used was 0,47 t/ha (IPCC 2006 GL, V4, CH4, Table 4.3).

Structural biodiversity analyzes were made using Gini (Gini, 1912) and Camino (Camino, 1976) indexes, and the graphic representation was attained using Lorenz curve (Lorenz, 1905). Gini biodiversity index, unlike other indexes, has a high quality in describing the structural diversity, (Cenusa, 2002, Lexerod and Eid, 2006, Roibu, 2008, Duduman, 2011, Klopcic and Boncina, 2011). Lorenz curve is a good indicator, used for describing the equitability of stands (Studeny et all, 2011, Valbuena, 2012).

To determine heterogeneity were also used other structural indexes like Shannon (Shannon, 1948) and Evenness (Pielou, 1969) using package BiodiversityR (Kindt and Coe, 2005) from RStudio program. The experimental dead wood (DW) volume distribution was computed using the program Mathwave EasyFit. In order to compare the experimental values with the theoretical ones were used Exponential, Gamma, Lognormal and Weibull functions and the goodness-of fit was tested using hi square criterion (χ^2), Kolmogorov Smirnov (KS) and Anderson-Darling (AD) tests.

RESULTS

Relationship between dead wood and alive trees

The presence of dead wood in virgin forest is very important and to highlight its role it was correlated with alive trees (Table 2).

Table 2. Relation between dead wood and alive trees

Research Plot	Volume, m^3						% DW from AT		
	Dead wood (DW)			Alive trees (AT)					
	Coniferous	Beech	Total	Coniferous	Beech	Total	Coniferous	Beech	Total
Șapte Izvoare	50,060	4,876	54,936	743,112	162,342	905,454	7	3	6
Penteleu – Viforâta 1	65,001	7,350	72,351	835,212	298,311	1133,523	8	2	6
Penteleu – Viforâta 2	96,891	26,458	123,349	446,614	357,218	803,831	22	7	15
Total	211,952	38,684	250,636	2024,938	817,871	2842,808	37	12	27
Mean	70,651	12,895	83,546	674,979	272,624	947,603	10	5	9

In all research plots, most of dead wood comes from coniferous species with a volume between 50.060 $m^3 \cdot ha^{-1}$ (Șapte Izvoare) and 96.891 $m^3 \cdot ha^{-1}$ (Penteleu – Viforâta 2). Beech dead wood volume is between 4,876 $m^3 \cdot ha^{-1}$ (Șapte Izvoare) and 26,458 $m^3 \cdot ha^{-1}$ (Penteleu – Viforâta 2), less significant than dead wood derived from coniferous. Total volume of dead wood it varies between 54,936 $m^3 \cdot ha^{-1}$ (Șapte Izvoare) and 123,349 $m^3 \cdot ha^{-1}$ (Penteleu – Viforâta 2), with an average of 83,546 $m^3 \cdot ha^{-1}$. The percentage of DW from AT for coniferous is between 7% and 22 %, with an average of 10%, while for beech is between 2% and 7% with an average of 5%. The total percentage between dead wood and alive trees varies between 6% and 15%, having an average of 9%.

Descriptive statistics of volume for dead wood

Number of samples of dead wood is between 63 (Penteleu – Viforâta 2) and 237 (Penteleu – Viforâta 1)(Table 3). Analyzing the results, it may be observed that lowest volume value of dead wood is 0,003 cubic meters (Penteleu – Viforâta 1) while the highest volume value is around 17 cubic meters (Penteleu – Viforâta 2 -volume which corresponds to a piece of lying dead wood having 104 cm breast diameter and 34 m length). The Șapte Izvoare research plot has the lowest volume value of dead wood (54,936 m^3) while Penteleu – Viforâta 2 registers the highest volume value of dead wood (123,349 m^2). The coefficient of variation is between 1,588 (Șapte Izvoare) and 3,076 (Penteleu – Viforâta 1), and standard deviation is between 0,364 (Șapte Izvoare) and 3,240 (Penteleu – Viforâta 2).

Table 3. Descriptive statistics of volume of dead wood

Research Plot	number of values	Min	Max	Range	sum	median	mean	Var	std.dev	coef.var
Șapte Izvoare	218	0.005	3.059	3.054	54.936	0.091	0.229	0.132	0.364	1.588
Penteleu - Viforâta 1	237	0.003	8.210	8.208	72.351	0.049	0.305	0.882	0.939	3.076
Penteleu - Viforâta 2	63	0.052	17.390	17.338	123.349	0.643	1.958	10.498	3.240	1.655

Dead wood volume in relation with decay class

Depending on the category of decay (Table 4), in Șapte Izvoare and Penteleu – Viforâta 2 research plots it may be observed that most of dead wood falls into the category *Snag 2* (Standing tree, died a while ago, without top and branches and also without bark or less bark remained) (Table 2), whereas in Penteleu Viforâta 1 most of dead wood volume falls in *Log 1* (Lying dead wood, still solid, with bark or beginning to lose bark).

Fitting of experimental DW volume distribution

To compare experimental and theoretical values Exponential, Gamma, Lognormal and Weibull theoretical functions were used (Figure 2).

Table 4. Dead wood volume according to decay class

Dead wood category	Volume, m^3			Mean
	Șapte Izvoare	Penteleu – Viforâta 1	Penteleu – Viforâta 2	
Snag 1	11,181	12,701	13,658	12,513
Snag 2	25,582	29,060	91,513	48,718
Log 1	17,532	30,590	-	16,041
Log 2	0,641	-	18,178	6,273
Total	54,936	72,351	123,349	83,545

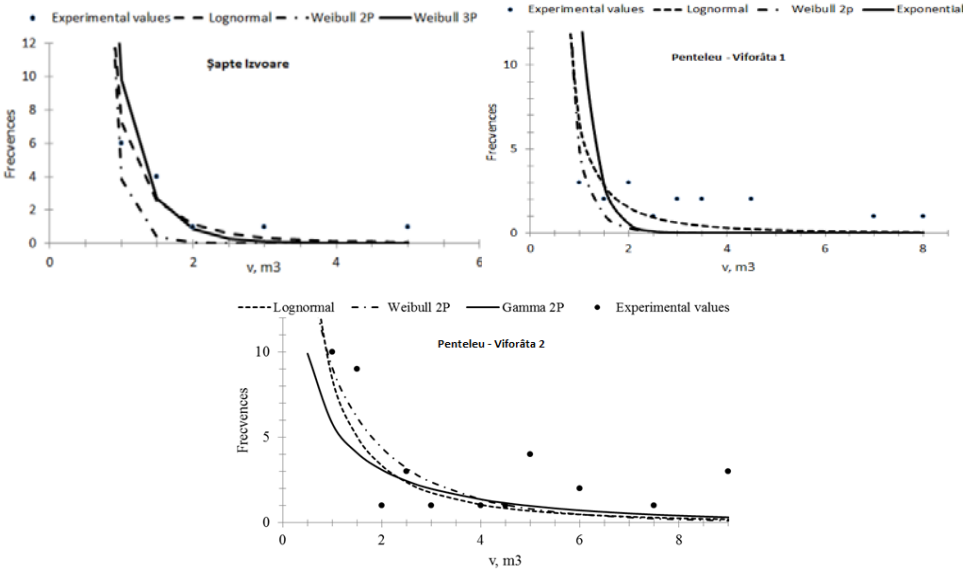

Figure 2. Dead wood volume distribution adjusted with theoretical functions

In order to determine which theoretical frequencies law is most suitable for describing this type of distribution the goodness of fit was tested, using χ^2 criterion, K-S and A-D tests (Table 5).

Table 5. Experimental values of specific goodness-of-fit tests

Research plot	Distribution	Kolmogorov Smirnov Test KS)		Anderson Darling Test (AD)		χ^2 Criterion (χ^2)	
		Experimental Values	Theoretical Values	Experimental Values	Theoretical Values	Experimental Values	Theoretical Values
Șapte Izvoare	Lognormal	0,069	0,092	1,086	2,501	5,474	14,067
	Weibull 2P	0,129	0,092	5,329	2,501	22,546	14,067
	Weibull 3P	0,099	0,092	3,194	2,501	39,084	14,067
Penteleu Viforâta1	Lognormal	0,061	0,088	1,074	2,501	8,819	14,067
	Weibull 2P	0,116	0,088	7,498	2,501	15,561	14,067
	Exponential	0,409	0,088	104,92	2,501	283,07	14,067
Penteleu-Viforâta2	Lognormal	0,07	0,168	0,383	2,501	0,837	5,991
	Weibull 2P	0,101	0,168	1,471	2,501	5,473	5,991
	Gamma 2P	0,212	0,168	3,011	2,501	0,018	5,991

Estimating dry biomass and CO_2 sequestration from dead wood

For all research plots dry biomass and CO_2 stock were obtained using allomeric equations (Table 6) related to dead wood volume.

Table 6. Values of dry biomass and CO_2 estimated

Research Plot	DW volume $m^3 \cdot ha^{-1}$	Dry biomass tones $\cdot ha^{-1}$	CO_2 stock tones $\cdot ha^{-1}$
Șapte Izvoare	54,93	19,65	9,23
Penteleu – Viforâta 1	72,35	24,12	11,34
Penteleu – Viforâta 2	123,34	29,91	14,06
Average	83,54	24,56	11,54

Quantity of dry biomass is between 19.65 tones $\cdot ha^{-1}$ and 29.91 tones $\cdot ha^{-1}$ with an average of 24.56 tones $\cdot ha^{-1}$. The dead wood from research plots sequesters approximatively a quantity on CO_2 ranging from 9.23 tones $\cdot ha^{-1}$ to 14.09 tones $\cdot ha^{-1}$, with an average of 11.54 tones $\cdot ha^{-1}$.

Analyzes of structural biodiversity

Structural biodiversity it is an important component of virgin forests and Lorenz curve succeeds to represent this in a graphical way (Figure 3).

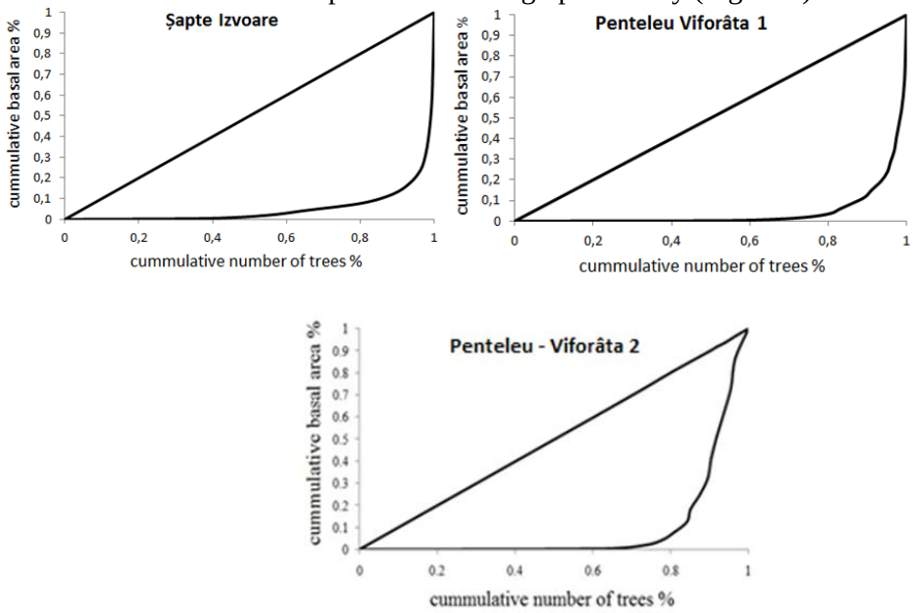

Figure 3. Lorenz curve for research plots Șapte Izvoare, Penteleu – Viforâta 1, Penteleu – Viforâta 2

Collected data from research plots were verified using special tests to indicate the level of heterogeneity. The values for G index from research plots are between 0.69 and 0.84 and the values for H index are between 1.62 and 1.74 (Table 7).

Table 7. Values of G and H indexes for testing structural homogeneity

Research Plot	Gini index (G)	Camino index (H)
Șapte Izvoare	0,69	1,71
Penteleu – Viforâta 1	0,71	1,62
Penteleu – Viforâta 2	0,84	1,74

To describe structural biodiversity using diameter data, were calculated indexes like Evenness and Shannon, which are the most popular among all alternative indexes (Valbuena et al., 2012). In our research plots, Shannon index (SH) is between 2.69 and 2.80, and values of Evenness index (E) is between 0.78 and 0.81 (Table 8).

Table 8. Values of E and SH indexes for testing structural homogeneity

Research Plot	Shannon index (SH)	Evenness index (E)
Șapte Izvoare	2,77	0,81
Penteleu – Viforâta 1	2,80	0,82
Penteleu – Viforâta 2	2,69	0,78

DISCUSSION

The large number and quantity of dead wood samples highlight a high heterogeneity of the researched stands. The amount of dead wood volume mentioned in literature falls into a range between 50 m^3 ha^{-1} and 200 m^3 ha^{-1} (Jedicke, Scherzinger, 1996). The high heterogeneity of dead wood is indicated by low values of standard deviation and the variation coefficient. Regarding the dead wood distribution according to the decay class, it may be observed that the forest stands weren't influenced by anthropic activities, dead wood was never harvested. The presence of considerably quantities of dead wood in different decay stages stabilizes the ecosystems and are providing food and habitats for many species as well as maintaining the health status (Tomescu et al., 2011).

Distribution of dead wood volume has a descending trend, with high values at small categories and a shape of reverse "J" (Westphal et al, 2006) characteristic for virgin stands. Goodness of fit for Șapte Izvoare and Penteleu - Viforâta 1 plot shows that all the conformity tests, indicate that the most suitable functions is Lognormal. Testing experimental values of Penteleu-Viforata 2 with theoretical function Lognormal, Gamma and Weibull, the $\chi 2$ criterion shows that all functions studied are suitable for describing the stand.

Also, Kolmogorov-Smirnov test show no differences between experimental and theoretical distribution for Lognormal and Weibull functions.

The relationships between dead and alive trees are influenced by the development stage of the stand, and the natural factors like wind, snow and insect's attacks. For all researched plots, most dead wood comes from coniferous, fact explained by the physiological age differences of beech and coniferous. The quantity of DW is important because it maintains the entomologic balance

between predators and parasites (Tomescu, 2011, 2013) and in this way, is demonstrated the superiority of virgin forests.

The carbon stock from dead wood, determined in our research, is higher than the one reported by Karankina et. al (2002), from Russian boreal forests (0.1-0.7 tones • ha^{-1}). Comparative to other research of virgin forests, the quantity of stocked carbon in dead wood fall in the lower limit of the interval, respective from 1.6 to 64.4 tones • ha^{-1} (Merganicova and Merganic, 2010), highlighting the high capacity of virgin forests in absorbing carbon.

The heterogeneity of researched plots is high, fact demonstrated by the large area between Lorenz curve and Equality line, specific to uneven aged stands and implicit to virgin stands. Also, Equality line represents a reference point for studying forest dynamics and stand development (Valbuena et al, 2012).

In our research, values of G index are higher than the lower limit (> 0.51) found in other research (Duduman, 2011, Valbuena et al., 2012) and close to maximum in others (Chivulescu et al., 2014). Regarding the coefficient of heterogeneity H for uneven aged stands, this was established to be between 1.3 and 2.8 (Ozcelik, 2009). The obtained values of H index in our research plots (1,62-1,74), demonstrate that the diversity is high and the stands have the characteristics of virgin uneven aged forests.

Shannon index has been used widely in the past for describing heterogeneity in ecological studies (LeMay and Staudhammer, 2005, Barbeito and Cañellas, 2009, Ercanli and Kahriman, 2015). Values between 0.0 and 2.30 for Shannon index are specific to uneven aged forests (Ercanli and Kahrimon – 2015, Roibu – 2010). In all researched plots the structural heterogeneity is high, fact demonstrated by the values of Shannon index (the obtained values are between 2.07 and 2.91.

CONCLUSIONS

This research highlights the capacity of virgin forests to maintain the stability of stands by its biodiversity. Biodiversity also maintained by the quantity of dead wood which provides food and habitats for many species. The presence of dead wood, in different decay stages, indicates that the ecosystems haven't been influenced by anthropic activities.

The dead wood distribution has a descending trend and the most suitable function to describe this type of ecosystems, in most cases, was Lognormal theoretical function. Also, was demonstrated that the stage development of stand and natural influences like snow, wind and insect attacks, influence the relationship between dead and alive trees.

The quantity of carbon stocked in dead wood was higher than the one registered in other research.

The high heterogeneity of the researched stands is demonstrated by the large area between Lorenz curve and Equality line, which is specific to uneven aged stands, but also by the values obtained using Gini, Camino, Evenness and Shannon indexes.

The most important fact demonstrated in this research is that the virgin forests are superior to managed forests and preserving this type of ecosystems is very important to humankind.

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Ekrem ÖGÜR, Levent ÜNLÜ, Fadime CANBULAT [1]

NEW RECORD FOR SPOTTED WING DROSOPHILA, *Drosophila suzukii* (MATSUMURA, 1931) (DIPTERA: DROSOPHILIDAE) IN KARAMAN, CENTRAL ANATOLIA

SUMMARY

Spotted wing drosophila, *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) is a polyphagous, invasive pest of many fruit crops. Although its native to East Asia, the pest has rapidly spread its range in many parts of the Americas and Europe since the first record in 2008. *Drosophila suzukii* was first recorded on strawberry crops in Erzurum, Eastern Turkey in 2014. This study was conducted in order to establish the presence of *D. suzukii* on cherry in Karaman. For this purpose, six apple cider vinegar traps were placed in three cherry orchards during period April 30th to June 25th 2017 and *D. suzukii* was confirmed in all surveyed locations. Totally, 39 *D. suzukii* adults (24♀ and 15♂) were captured in the traps during the monitoring period on cherry orchards. This paper presents a new record of the occurrence of *D. suzukii* in Karaman (Ermenek), Central Anatolia on cherry. Also this is the first report of the presence of *D. suzukii* in cherry grown in Turkey.

Keywords: Central Anatolia, cherry, *Drosophila suzukii*, invasive pest, spotted wing drosophila, Turkey

INTRODUCTION

Drosophila suzukii (Matsumura), also known as Spotted Wing Drosophila in the United States, and Cherry Drosophila in Japan, is an invasive, highly polyphagous and a devastating pest that originates from East Asia (Kanzava, 1935; Walsh et al., 2011). It was first described by Matsumura in Japan in 1931. The first record outside of Asia was in Hawaii in 1980 (Hauser, 2011), then in California in 2008 *D. suzukii* was found for the first time (Hauser, 2009). In Europe, it was first reported in Spain and Italy in 2008 (Grassi et al., 2011; Raspi et al., 2011; Calabria et al., 2010; Cini et al., 2012). Since then, the geographical distribution of *D. suzukii* has spread rapidly and many other European countries made their first record (Figure 1).

Adults of *D. suzukii* are small flies approximately 2.25–4.0 mm long (females are usually slightly larger than males), with red eyes, yellowish brown thorax and dark brown abdomen with black transverse stripes. Males display a black spot on the outer edge of each wing and two sex combs on the first and

[1] Ekrem Ögür, (corresponding author: ekremogur@selcuk.edu.tr), Levent Ünlü, Fadime Canbulat, Selcuk University, Faculty of Agriculture, Department of Plant Protection, Konya, 42250, TURKEY,

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second segments of their front legs, females possess a large serrated ovipositor, clear wings without spots and lack sex combs on legs (Hauser, 2011; Walsh et al., 2011; Cini et al., 2012; EPPO, 2013). Even though males and females of *D. suzukii* can be easily detected by these distinguishing features of the two sexes, similar characters are present in some other closely related *Drosophila* species, such as *Drosophila subpulchrella*, *Drosophila pulchrella*, *Drosophila immacularis* and *Drosophila immigrans* (Hauser, 2011; Cini et al., 2012; EPPO, 2013 Asplen et al., 2015).

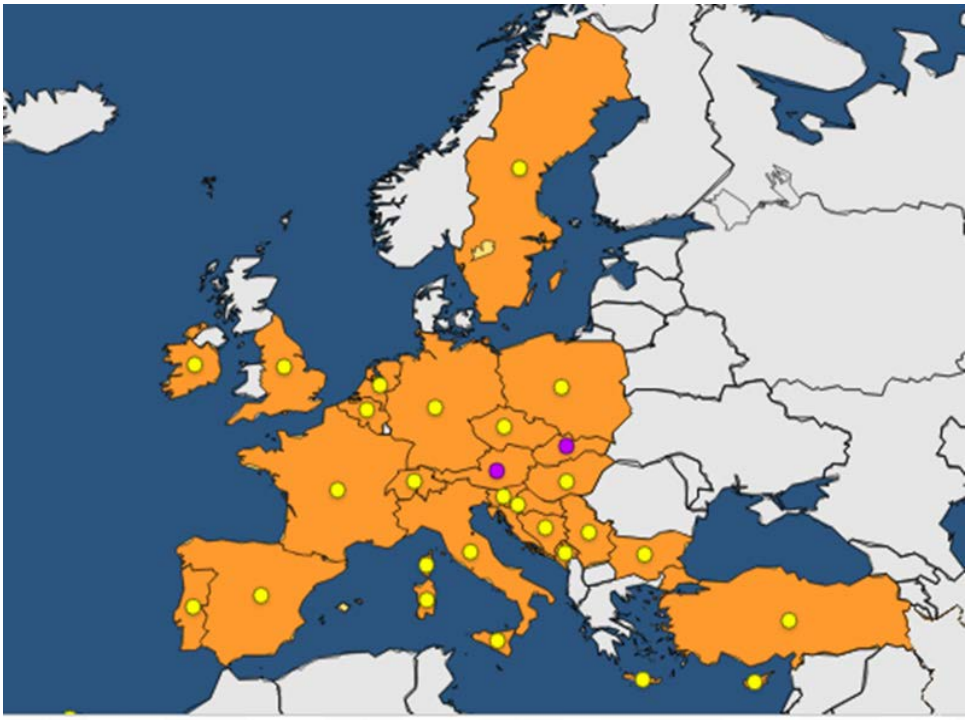

Figure 1. Distribution map of *Drosophila suzukii* in Europe (EPPO, 2017). France (NPPO of France, 2010), Slovenjia (Seljiak, 2011), Switzerland (NPPO of Switzerland, 2011), Belgium (NPPO of Belgium, 2011), Croatia (Milek et al., 2011), Austria (NPPO of Austria, 2012), Netherlands (NPPO of Netherlands, 2012), Portugal (NPPO of Portugal, 2012), Germany (Vogt et al., 2012), United Kingdom (EPPO, 2012), Hungary (Kiss et al., 2013), Slovak Republic (NPPO of Slovak Republic, 2014), Czech Republic (NPPO of the Czech Republic, 2014), Greece (Máca, 2014), Poland (NPPO of Poland, 2014), Bulgaria (NPPO of Bulgaria, 2014), Bosnia and Herzegovina (Ostojić et al., 2014), Serbia (Toševski et al., 2014), Ireland (NPPO of Ireland, 2015), Romania (Chireceanu et al., 2015), Montenegro (Radonjić and Hrnčić, 2015), Sweden (NPPO of Sweden, 2016), Turkey (Orhan et al., 2016) and the last record was from Cyprus (NPPO of Cyprus, 2017). (Legend: ○ Present, ● Transient).

Spotted Wing Drosophila is a member of the subgenus *Sophophora* (Diptera: Drosophilidae) (Asplen et al., 2015). Drosophilidae consists of approximately 4,200 species, but they are generally not considered as pests because they are primary consumers of microorganisms, yeasts, and bacteria associated with the early stages of plant decay (Deprá et al., 2014; dos Santos et al., 2017). Unlike the vast majority of *Drosophila* species, which oviposit in decaying or rotting fruit, *D. suzukii* females are able to lay eggs in undamaged, ripening fruit by its serrated ovipositor (Gargani et al., 2013; Mazzi et al., 2017). The oviposition wounds and internal larval feeding as well as the secondary pathogens make infested fruit unmarketable (Goodhue et al., 2011; Walsh et al., 2011). The pest has included on A2 list as a quarantine pest by the European Plant Protection Organization (EPPO, 2013).

This invasive pest observed most often on cherries, peaches, plums, persimmons, strawberries, grapes, apricots, blackberries, blueberries, figs, hardy kiwis, nectarines, pears, pluots, raspberries, apples, oranges, tomatoes and also been found on wild and ornamental plants by several authors (Hauser et al., 2011; Lee et al, 2011; Walsh et al., 2011). This economically important pest led to $500 million in annual losses in Western US production areas without adequate control measures (Goodhue et al., 2011).

The goal of this study was to check the presence of the spotted wing drosophila, *D. suzukii* on cherries in Karaman province. This paper provides the first report on the occurrence of *D. suzukii* in central Anatolia. Also this is the first report of the presence of *D. suzukii* in cherry grown in Turkey.

MATERIAL AND METHODS

Monitoring and Trapping of *Drosophila suzukii*

The monitoring of *D. suzukii* was conducted from the end of April (when the fruits were green) to the end of June (until the harvest) in 2017, in three cherry orchards which located in Ermenek, about 120 km south-west of Karaman (Central Anatolia). The cherry cultivars traditionally bred here are “0900 Ziraat”.

In order to detect the *D. suzukii*, two apple cider vinegar traps were placed on cherry trees in each orchard, a total of six traps. The location of the Orchard 1, Orchard 2 and Orchard 3 were 36°37′34″N 33°1′9″E, 36°36′7″N 32°56′2″E, 36°37′42″N 33°1′31″E, respectively. Each trap consisted of a 500 ml durable plastic bottles, baited with 100 ml of apple cider vinegar, with five holes 5 mm in diameter on the top sides, to allow the entry of the attracted flies (Figure 2) (Grassi et al., 2011; Gargani et al., 2013; Baser et al., 2015). Traps were hung at a height of 1.5-2 m on the south-eastern side of tree canopy (Grassi et al., 2011; Labanowska and Piotrowski, 2015; Radonjić and Hrnčić, 2015). Traps were replaced weekly with new ones and exposed traps were moved to laboratory where collected insects were preserved in 70% ethanol for further analysis (Grassi et al., 2011; Baser et al., 2015).

Figure 2. Apple cider vinegar trap on cherry tree

Identification of *Drosophila suzukii*

Morphological identification of the *D. suzukii* was done by using a stereomicroscope Olympus SZ61 following the Hauser (2011) and EPPO (2013) diagnostic protocol for *D. suzukii*. The males were distinguished by a conspicuous black spot on the outer edge of each wing and by the two sex combs on each of their front legs. Females were identified by large serrated ovipositor with many dark sclerotized teeth (Hauser, 2011; Deprá et al., 2014; Lue et al., 2017).

RESULTS AND DISCUSSION

The adults of *D. suzukii* monitoring was carried out at six traps in three locations in 2017. The presence of *D. suzukii* was confirmed in all inspected locations. Results associated with the trap counts from the three locations are presented in Table 1. Data in Table 1 show that a total of 39 *D. suzukii* adults (24♀ and 15♂) were captured in the traps during the monitoring period on cherry orchards in Ermenek. Females were captured at first and outnumbered males in all locations. Traps also captured other flies, thrips, earwigs and houseflies. Usually, the proportion of other insects in traps was very high than *D. suzukii*.

Table 1. Occurrence of *D. suzukii* adults captured by apple cider vinegar traps in cherry orchards in 2017

Orchards No	Collection date							
	07.05.17	14.05.17	21.05.17	28.05.17	04.06.17	11.06.17	18.06.17	25.06.17
1	1♀	-	1♀	1♀	-	1♀ 3♂	1♀	-
2	-	1♀	-	-	-	1♀ 3♂	2♀ 1♂	2♀ 1♂
3	-	-	-	-	4♀	3♂	4♀ 2♂	5♀ 2♂

In morphological sense, specimens of *D. suzukii* do not differ from the specimens of this species which previously recorded in other parts of Europe. Adults are 2-3 mm in length with red eyes and a pale brown to yellowish-brown thorax and abdomen. Males possessed a black spot on the outer edge of each wing (Figure 3a) and two sex combs on the first and second segments of their front legs (Figure 3c). Females slightly larger than males (Figure 3b) and possessed a distinctly serrated ovipositor (Figure 3d) which allows them to lay eggs under the skin of a healthy, ripening fruits. The wings of females were clear without spots and there were no sex combs on their feet. This morphological characters make easy the identification of *D. suzukii* and distinguish it from other *Drosophila* species.

Figure 3. General view of *D. suzukii* a) male, b) female, c) sex combs on the first and second segments of male front legs, d) serrated ovipositor of female

Drosophila suzukii was detected for the first time in Erzurum, Turkey on strawberry crops by Orhan et al. in 2014. Just three years after the first detection, we found the pest on cherry crops in Ermenek (Figure 4). Although there are approximately 1000 km between Erzurum and Ermenek, the definition of the pest in Ermenek should not be surprising. Because the pest spread 1400 km/year either passively or actively through infested fruits (Calabria et al., 2010). With this study, we presents a new record of the occurrence of *D. suzukii* in Ermenek

(Karaman), Central Anatolia on cherry and this is the first report of the presence of *D. suzukii* in cherry grown in Turkey.

Drosophila suzukii prefers a temperate climate (Walsh et al., 2011), like that of the many parts of fruit growing areas of Turkey. Therefore, spreading of the *D. suzukii* may lead to important economic costs in the absence of necessary measures.

This polyphagous pest threatened the fruit production and led to severe crop losses in its newly invaded areas in many parts of the world (Hauser, 2011; Lee et al., 2011; Walsh et al., 2011; Cini et al., 2012; Deprá et al., 2014). The adults of *D. suzukii* are very mobile so the pest has rapidly spread its geographic range in a very short time and became one of the most devastating pests, particularly on cherry and berry fruits and also on vineyards especially in the temperate regions (Saguez et al., 2013; Radonjić and Hrnčić, 2015).

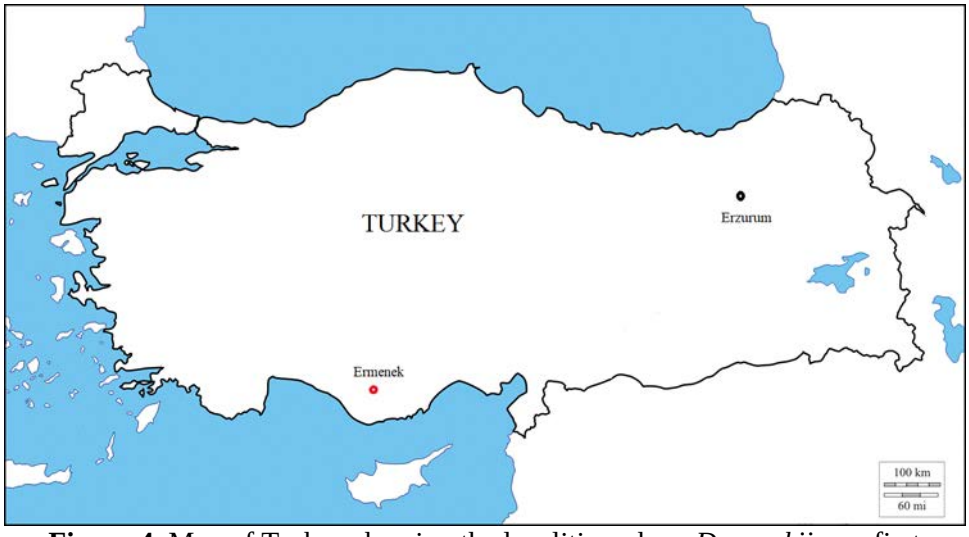

Figure 4. Map of Turkey showing the localities where *D. suzukii* was first reported on strawberry and cherry

CONCLUSIONS

This study is the first report on the occurrence of *D. suzukii* in cherry orchards in Ermenek (Karaman), Turkey. There are large cherry growing areas not only in Central Anatolia, but also in other regions of Turkey. In view of the rapid spread of the pest and potential for economic damage, further studies of *D. suzukii* biology, population dynamics and monitoring of the distribution should be done, especially in fruit growing areas with temperate climates in Turkey. Also, farmers should be informed about this new invasive pest.

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